HIPPOCAMPAL REPLAY AND LEARNING FROM REWARD

by

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Abstract

The hippocampus is necessary for the encoding and consolidation of new experiences, as well as implicated in retrieval of memories for planning and guiding behavior. Hippocampal pyramidal neurons, called place cells, are active in a position dependent manner and the population encodes the animal's current location as it moves around, exploring an environment. During pauses in exploration or subsequent sleep, place cells are reactivated in sequences, known as replay, that reflect trajectories through previous or current environments. Since replays are relatively difficult to detect, they are often studied indirectly through events in the local field potential called sharp wave ripples (SWRs). A key piece of evidence for the involvement of replay in consolidation is the increase in SWRs in the presence of reward. Blocking SWRs during behavior interferes with task performance, suggesting that retrieval is affected. Consequently, awake replay is considered a candidate mechanism of both consolidation and retrieval. We used high density tetrode recording to detect replays in behaving rats when reward was increased or removed. Replays that occurred in reverse, the opposite direction of experience, were increased when reward increased while forward replays did not change. We interpret these results as evidence that reverse replays are involved in learning about reward and consolidation while forward replays relate to functions such as memory retrieval or planning. The importance of reward in replay processing is further supported by preliminary results showing that replays occur during reward consumption at higher than chance levels. This data was obtained using a custom application for detecting licking behavior. Our results demonstrate an important difference between forward and reverse replays and emphasize necessity of considering replay content rather than

relying on SWRs. We present a preliminary novel program for real-time decoding of replay content, the Online Replay Decoding Algorithm (ORDEAL), which can be used to associate particular replay trajectories with rewarding brain stimulation or other manipulations. The research and technical advances described in this thesis represent a significant step towards understanding how replay interacts with reward, providing a foundation for the continued study of this important topic.

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Chapter 1: Background

Our brains are evolved to generate behaviors that will optimize our chances of survival. We select our actions based on our knowledge about the world and we are remarkably adept at adapting or changing our behavior to utilize new information about possible outcomes. Appropriate action selection requires learning, the process of acquiring new information, and memory, the information that is stored and can be recalled when it is needed. In this dissertation, I will discuss cell ensemble level mechanisms of memory consolidation and retrieval in the hippocampus and how these processes respond to a positive outcome, reward. This research provides insight into the neural basis of learning and memory, and in particular how memory of positive or negative outcomes is stored so that it can be used to guide future behavior.

In the lab, learning and memory is often studied in rodents and primates. In these models, single unit electrophysiology allows us to study the neural representations of the stimuli that compose experience. Simple stimulus representations exist in primary sensory cortices and are gradually built up to represent complex stimuli in high order cortical areas. The medial temporal lobe (MTL), including the hippocampus, receives inputs from the highest levels of sensory cortices as well as subcortical structures implicated in emotional responses. This connectivity suggests that the MTL may contain or coordinate the complete representation of an experience. Indeed the MTL is known to play a critical role in the process of memory encoding and consolidation.

Principal excitatory cells in the hippocampus are often called place cells because of their spatial tuning. An ensemble of place cells can represent an entire environment such as a room or maze, and a trajectory within the environment is encoded by the sequential activation of place cells with adjacent fields. Place cell sequences occur during spatial navigation, but are also reinstated during periods of sleep and immobility on a much faster timescale, a phenomenon known as replay. Of all neural correlates of experience observed in different parts of the brain, replay most explicitly represents entire episodes of experience. Furthermore, replays occur on a timescale conducive to the induction of synaptic plasticity and are important for spatial learning and memory.

Reward is a stimulus that reinforces behavior. Experimentally, this means that animals can be trained to perform tasks in exchange for reward, and they will seek reward if it is available. Reward is also associated with activity of the midbrain dopamine system. Dopamine enhances synaptic plasticity in the hippocampus and facilitates learning in a variety of experimental settings. This suggests that reward may have a significant effect on hippocampal function, in particular replay. I performed experiments to probe the interaction between reward and replay, the results of which I will present in this document (Chapter 3). I will also discuss two applications that I wrote to support these and similar studies and the insights gained from these developments thus far (Chapters 4 and 5). I hope that this work will expand our knowledge of how rewarding experiences are processed by the brain, and lead to better understanding of reward-seeking behavior in animals and humans.

Hippocampal function and anatomy

In 1953, Henry Molaison, known by the scientific community as patient HM, underwent an extensive surgery to bi-laterally remove large portions of his medial temporal lobe in an attempt to cure him of debilitating seizures. The seizures improved, but a surprising and devastating collateral effect occurred. The surgery caused severe anterograde amnesia. HM was unable to make new memories about anything that happened to him, anyone he met, or any information he was told from the time of his surgery (Scoville and Milner, 1957). The extent of HM's disability was carefully explored and cataloged, and eventually it was found that on certain tasks he was able to improve his performance across days, indicating the existence of a learning mechanism spared by his lesion (Corkin, 1968; Corkin, 2002). For example, tracing the outline of a star on a piece of paper while viewing one's hand in the mirror is awkward and difficult at first, but becomes easier with practice. This was true for patient HM despite his inability to remember the task from one session to the next (Corkin, 2002). The discovery of spared skill learning in patient HM led to our current understanding of the two kinds of learning: that which is dependent upon the hippocampus and surrounding MTL, and that which is not.

The two types of learning can be broadly defined as learning of skills and habits, and learning of facts and events (Cohen and Squire, 1980). Procedural memory includes the memory of, for example, how to ride a bike or play an instrument. These are skills acquired through practice, and may be difficult to explain or describe explicitly.

Procedural memory was spared in patient HM because it is not dependent on the MTL, but rather regions such as the striatum (McDonald and White, 1993). On the other hand, the second category of memory, known as declarative or explicit memory, is dependent on the MTL (Squire and Zola-Morgan, 1991). Declarative memory involves the explicit recall of facts or information, known as semantic memory, or the ability to recount an autobiographical experience or event, known as episodic memory. In this dissertation, I will discuss episodic memory and the potential mechanisms by which it is encoded and consolidated during experience, or recalled during decision making.

Within the MTL, the hippocampus is critical for episodic memory. The region removed in HM's surgery included most of the anterior hippocampus as well as surrounding entorhinal cortex (EC) (Corkin et al., 1997; Scoville and Milner, 1957; Squire and Zola-Morgan, 1991). Even human patients with lesions that are limited to the hippocampus display deficits in memory and imagination. While the patients can recall facts from the news or their lives, they have difficulty recounting episodes of their lives or creating imaginary scenarios. For example, a patient may be able to list items on a beach (sand, a boat, a seagull), but has trouble integrating these items into a scene or describing their locations relative to the narrator (Hassabis et al., 2007). This suggests that the hippocampus supports remembering and imagining experiences, perhaps by binding semantic components into a cohesive context.

The context of an episode is inherently based in its location, and space provides a continuous framework underlying experience. Therefore it is not completely surprising

that in addition to episodic memory, the hippocampus is crucial for spatial learning and memory (Barnes, 1988). Spatial memory in rats is commonly tested using the Morris water maze. In this test, rats search for a hidden underwater escape platform while swimming in an opaque pool. Spatial learning of the platform location is measured by escape latency over a series of trials or the time spent in the platform area during a probe trial in which no platform is available. Hippocampal lesioned rats are impaired in the Morris water maze compared to their intact counterparts (Morris et al., 1982; Sutherland et al., 1983). This suggests that the same system that supports spatial memory is also capable of integrating objects and events into episodic memory in the hippocampus (Buzsaki and Moser, 2013).

Contextual information flows into the EC from other high order cortical areas, with spatial inputs targeting the medial EC, while the lateral EC receives object and place related inputs (Hargreaves et al., 2005; Kerr et al., 2007). The classic hippocampal circuit is the tri-synaptic pathway from the superficial layers of EC to dentate gyrus, to CA3, and finally to CA1, although the CA fields also receive direct inputs from EC. Sparse coding in the dentate gyrus is thought to perform pattern separation on EC input, allowing for the ability to discriminate between two similar environments (Leutgeb et al., 2007). The recursive CA3 network could give rise to attractor dynamics to support pattern completion, allowing recognition in the presence of incomplete information (Neunuebel and Knierim, 2014; Rolls and Treves, 1990). CA1 in turn sends outputs to layer V of EC both directly and via the subiculum (Amaral and Witter, 1989; Sasaki et al., 2015).

The hippocampal formation processes information in one of two states defined by the oscillatory behavior of the local field potential (LFP). The theta state, characterized by strong 6-10 Hz oscillatory activity, is associated with exploration, movement, and whisking in awake rodents and occurs during the rapid eye movement stage of sleep (Buzsaki, 2002; Colgin, 2013). Theta oscillations are driven by inputs from the medial septum, probably including both cholinergic and GABAergic neurons (Brandon et al., 2011; Toth et al., 1997; Vandecasteele et al., 2014). On the other hand, while animals are eating, drinking, grooming, resting, or during slow wave sleep, the hippocampus displays large irregular activity. During this state, bursts of cell firing are reflected in brief oscillatory events in the range of 150-250-Hz called sharp wave ripples (SWRs) because the fast ripple oscillation rides on a large deflection, the sharp wave, typically lasting 50-300-ms (Buzsaki, 1986). Sharp waves are thought to originate in CA3 from a build-up of excitatory activity within the recurrent network. The resulting bursts of activity propagate to CA1 upon brief release of inhibition, generating ripples (Colgin, 2016). Thus, it has been proposed that the exploratory theta state in awake animals is necessary for encoding new experience, while the sharp wave ripple state supports offline mechanisms such as memory consolidation (Buzsaki, 1989).

Place coding in the medial temporal lobe

While lesion studies implicated the hippocampus in spatial learning, another line of evidence for this function was uncovered with single unit recording. In 1971, John

O'Keefe and John Dostrovsky identified 8 cells in the dorsal hippocampus which coded for space. Unlike cells in primary sensory cortices, these cells were not driven by any single sensory stimulus in the recording room. Instead activity of these cells peaked when the rat was in a certain location and facing a certain direction in the experimental arena, for which they were named place cells. O'Keefe and Dostrovsky interpreted the activity of place cells as an internal representation of the environment (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971), equivalent to the cognitive map proposed over 20 years earlier by psychologist E. C. Tolman (Tolman, 1948).

The specific location in a given environment where a place cell is active is called the place field. Place fields come in variable sizes and shapes, but once formed they are stable unless perturbations to the environment are introduced. For example, when the walls of an arena are changed from rectangular to circular, place cells either change their fields or lose their fields entirely in the new context (Muller and Kubie, 1987). Meanwhile, cells that were previously silent may begin to express fields. Total redistribution of place fields is called global re-mapping as opposed to rate remapping, in which field location is stable but rates are changed, or partial remapping in which some cells remap but others do not (Colgin et al., 2008).

Place cells in CA1 and CA3 have different tendencies to re-map which are attributable to their different internal and external connectivity. CA3 generates distinct place codes of similar arenas, while CA1 representations of similar arenas tend to overlap (Leutgeb et al., 2004). The more extensive re-mapping in CA3 may be necessary because of the

attractor characteristics of the network in which similar representations could easily converge. At the same time, the CA3 population behaves more coherently than CA1 when local and distal cues are rotated relative to each other (Lee et al., 2004). The relative stability of the CA3 representation suggests that pattern completion may be performed on a subset of the rotated cues generating a representation similar to the original environment. The hippocampus could then compare the previously experienced environment represented in CA3 to the CA1 representation, which is based on current inputs, perhaps through direct projections from the EC. This comparison can be used to perform novelty detection (Lisman et al., 2011).

During exploration, place cells are activated as the animal travels through consecutive place fields. However, fields are overlapping and thus the spike trains of multiple cells overlap during behavior. Interestingly, the overlapping firing of cells is structured within cycles of the theta rhythm observed during running. Within each cycle, the place cells fire in short sequences, starting with cells whose field centers are slightly behind the animal's location, and ending with cells whose fields are slightly ahead of the animal. These sequences are known as theta sequences (Dragoi and Buzsaki, 2006; Foster and Wilson, 2007). Theta sequences are likely to be important for memory encoding, because they occur during the theta state and they represent segments of the rat's experience.

Other representations of space exist in the medial temporal lobe, which contribute to the expression of place fields. In 2005, Hafting and colleagues, recording in the superficial

layers of the medial EC, discovered cells which were active throughout a two dimensional environment at regular intervals arranged like the nodes of a hexagonal grid (Hafting et al., 2005). This patterning gave rise to the name grid cells. Grid cells are organized into modules of increasing grid size along the dorsal-ventral axis of the EC (Stensola et al., 2012). The EC also contains cells that respond to borders in an environment, such as the side of the arena, or a wall protruding into the arena (Savelli et al., 2008; Solstad et al., 2008). Border cells may serve as an anchor or reference point by which grid cells align their grids. Grid orientation may also be influenced by head direction cells which respond to the orientation of the animal's head regardless of its position and are found in both cortical and subcortical regions including the EC (Sargolini et al., 2006), the presubiculum (Taube et al., 1990), and the anterior thalamic nuclei (Taube, 1995).

Inputs from multiple overlapping and differently oriented grid cells could converge on a place cell, generating the representation of a single location. However, disruption of grid cells by medial septum inactivation does not impair place fields (Brandon et al., 2014) while reversible inactivation of the dorsal hippocampus using the GABA receptor agonist, muscimol, leads to temporary loss of grid-like activity in grid cells (Bonnevie et al., 2013). Still, place cells do receive inputs from spatially and non-spatially tuned cells in the EC (Zhang et al., 2013). It is likely that these inputs combine with local hippocampal mechanisms to generate place fields.

Hippocampal replay

When a large number of place cells are simultaneously recorded, it is possible to find place fields tiling an entire environment. A result of this complete representation is that as a rat moves through the environment, passing through adjacent place fields, place cells are activated in a sequence that is uniquely determined by the particular trajectory taken. Thus, if place field locations are known, it is possible to determine the position of an exploring animal simply by observing the sequential activity in the hippocampus (Wilson and McNaughton, 1993). However, spatial memory requires the ability to store and recall sequences of positions that are not currently being experienced. Furthermore, planning may invoke an element of imagination, the ability to activate sequences of positions that may not have been explicitly visited, at least in that particular order. The simple firing of place cells within their fields is not sufficient for these kinds of mental traversal of the environment.

Since the discovery of place cells, it was observed that the cells sometimes fired outside of their fields while the rat ate, drank, or was immobile in the quiescent or SWR associated state (O'Keefe, 1976). Furthermore, place cell activity was identified after experience while rats were sleeping in a small enclosure, separate from the arena. Wilson and McNaughton showed that place information re-activated in post-experience sleep contained the structure of the previous experience (Wilson and McNaughton, 1994). They calculated the cross-correlations of the spikes trains for all pairs of cells during a run session and during pre- and post-run sleep sessions. Correlations between cells with overlapping place fields were high during the run, and persisted in post-run

sleep. The same cell pairs were not highly correlated during the pre-run sleep, indicating that an experience dependent change in connectivity had occurred. These pair-wise correlations in post-run sleep were even higher when considering the bursts of spiking in SWRs only.

The discovery that place cells with overlapping fields were co-active during post-run sleep led to the question of whether the temporal order of cell activity (which cell fired first) was preserved during sleep. Rats ran in loops in one direction around a continuous track on which cells with adjacent fields would fire only in one order. This study showed that the temporal order was preserved in post-run sleep relative to the baseline pre-run sleep (Skaggs and McNaughton, 1996) although the method used is susceptible to artifactual results due to misclustered spikes (Quirk and Wilson, 1999). Extending the pairwise analysis to multiple cells, Lee and Wilson showed that during activity bursts coinciding with SWRs during post-run slow wave sleep, place cell ensembles activated in the order of their fields at higher than chance levels (Lee and Wilson, 2002). This ordered activity is now commonly referred to as sequential re-activation, or replay.

Although they were discovered during sleep, replays also occur in awake animals during quiescent SWRs (Foster and Wilson, 2006). Through studies of awake replays we have begun to understand the characteristics and limitations of replay. Most awake replays are local; they represent the current environment and tend to begin at the current location. However, they can also represent remote environments such as a track that the rat experienced earlier in the day (Karlsson and Frank, 2009). Experience in an

environment is necessary for replays to develop (Silva et al., 2015), but only a small amount of experience is necessary (Wu and Foster, 2014). Furthermore, the amount of experience a rat has with a particular trajectory or how recently the trajectory was experienced does not predict the frequency with which that trajectory will be replayed (Gupta et al., 2010). SWR duration does not necessarily limit the length or distance covered by a replayed trajectory. Large environments can be replayed over trains of SWRs, which tend to segment trajectories by environmental landmarks (Davidson et al., 2009; Wu and Foster, 2014). Replays can represent different possible trajectories on a maze with a choice point (Wu and Foster, 2014), or in an open field (Pfeiffer and Foster, 2013). Together, these experiments show that replays quickly and flexibly represent experienced environments, but their content and occurrence are unpredictable by basic measures such as local sensory inputs or behavior.

Since place field firing is direction as well as position dependent, replays also have a distinct heading direction component. Foster and Wilson described replays in which cells fired in the reverse order as they were active during a lap across the linear track, that is, as if the rat were moving backwards along the track (Foster and Wilson, 2006). Reverse replays occur interspersed with forward replays, although Diba and Buzsaki observed more reverse replays following a run across the track and more forward replay preceding a run (Diba and Buzsaki, 2007). Reverse and forward replays have sometimes been observed in different amounts in the same environments (Davidson et al., 2009; Diba and Buzsaki, 2007; Wu and Foster, 2014), which may be related to the tendency of reverse replays to occur more often in novel environments (Foster and Wilson, 2006).

Since its discovery, replay has been considered a candidate mechanism for memory consolidation. First, it occurs during SWRs in which information may be transferred for long term storage in the neocortex (Buzsaki, 1989). Further, the memory consolidation theory is intuitive because replay is a neural representation of an experience, which is revisited after the experience takes place. Through this reactivation, synaptic strengthening within the population could occur in the absence of additional experience. To support this hypothesis, the speed of replay is consistent with the timescale of induction of spike timing dependent plasticity (Bi and Poo, 1998). The induction of plasticity could occur not only within the hippocampal circuit, but also downstream in the neocortex for long term memory storage. In fact, sequential activity has been observed simultaneously in the hippocampus and visual cortex (Ji and Wilson, 2007) and the medial prefrontal cortex (Euston et al., 2007; Peyrache et al., 2009) during sleep. There are limited methods for causal studies of SWR function, but evidence has been obtained by terminating SWRs (and presumably replay) upon their onset by using ripple power-triggered electrical stimulation of the ventral hippocampal commissure. SWR interference during post training sleep impairs spatial memory indicating a disruption in the consolidation process (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009).

The consolidation hypothesis of replay function is challenged by multiple lines of evidence implicating replay in imagining or planning future routes. Awake replays have been shown to represent never taken "shortcuts" in which two independently

experienced trajectories were combined into a novel trajectory, which was not actually experienced by the animal (Gupta et al., 2010). Blocking SWRs during an alternation task specifically impairs the working memory component of the task, which could be due to disruption of either planning of future trajectories or retrieval of the most recent trajectories (Jadhav et al., 2012). When rats pause at a choice point and look down their possible future paths (vicarious trial and error) short sequences travelling forward along the possible routes have been observed which were interpreted as mental "looking ahead" for route planning and predicting future outcomes (Johnson and Redish, 2007). Consistent with this idea, more activation is observed before correct as opposed to incorrect trials in an alternation task (Singer et al., 2013). Finally, in rats performing goal directed navigation in an open field, replays occurring away from the known goal location represent possible routes to that location (Pfeiffer and Foster, 2013). In this experimental setting, replays often represent novel trajectories and are predictive of the animal's future behavior.

Ample evidence exists to support the notion that awake replay in particular may be a mechanism of both memory consolidation and memory retrieval for planning. These functions are not exclusive. Indeed the hippocampus is known to be important for both, as seen in the hippocampal lesion patients discussed earlier. In Chapter 3 I will discuss a possible way that hippocampal replay could accomplish both functions to support learning and planning.

Interaction with reward, dopamine, and plasticity

Many studies of spatial memory involve learning reward locations in an environment. Interestingly, reward itself modulates place cell activity and replay, perhaps as a way of facilitating remembering the reward location. For example, it has been shown that a subset of place fields re-map slightly to represent reward locations (Dupret et al., 2010). Additionally, place field stability across laps is modulated by reward contingency, specifically more stability is observed during a more difficult task (Wikenheiser and Redish, 2011) consistent with an increase in attention (Kentros et al., 2004). Replays also tend to converge on known reward locations (Pfeiffer and Foster, 2013). In the most direct study of reward effect to date, Singer and Frank showed that SWRs and place cell reactivation are increased in the presence of reward and the effect is further enhanced during learning of a new reward contingency (Singer and Frank, 2009).

Spatial learning and replay development depend on the induction of synaptic plasticity. In hippocampal pyramidal cells a class of glutamate receptors, called NMDA receptors, mediate synaptic plasticity. Strong activation of NMDA receptors leads to long term synaptic strengthening, or potentiation, while weak activation leads to long term synaptic weakening, or depression. Infusion of an NMDA receptor antagonist, AP-V, impairs spatial learning over consecutive training days on the water maze task (Morris et al., 1986). In a newer study, global injection of the NMDA receptor antagonist CPP did not impair the initial learning of goal locations in an open arena, but did impair later recall of those locations (Dupret et al., 2010). Place fields are fairly stable under a similar drug, CPPene, while replays do not develop under this blockade (Silva et al., 2015).

Interestingly, this study found that replays of environments experienced before CPPene injection remained robust after the injection. This indicates that the formation but not the maintenance of replay is dependent on NMDA receptor mediated synaptic plasticity. Furthermore, blocking SWRs (including replays) after learning results in a "rebound" increase in the rate of SWRs, which is blocked under NMDA receptor blockade with MK-801 (Girardeau et al., 2014). Together these studies suggest that the learning deficit observed under NMDA receptor blockade may be due to the rat's inability to replay the environment.

Recordings in hippocampus and reward related areas support the interaction between reward and replay. The ventral striatum receives inputs from the hippocampus as well as dopaminergic neurons in the ventral tegmental area, and is central to reward association processing. Similar to hippocampal neurons, ventral striatal neurons display experience dependent reactivation in post experience sleep and a subset of these neurons are modulated by hippocampal SWRs (Pennartz et al., 2004). In a dual hippocampal and ventral striatal recording experiment it was shown that pairwise reactivation between hippocampal and striatal neurons was greatest in pairs in which the hippocampal neuron's firing preceded the striatal firing during behavior (Lansink et al., 2009). This was interpreted as evidence for the reactivation of reward associations driven by hippocampal activity. Reward responsive ventral tegmental area neurons were also found to be active during hippocampal SWRs during awake quiescence, resulting in coordination of dopamine release and replay to support reward learning (Gomperts et al., 2015). Rewarding place cell activation during sleep alone has been

shown to be sufficient for the development of behaviorally measured place preference indicating a functional role for simultaneous place cell and reward circuit activity (de Lavilleon et al., 2015).

Synaptic plasticity, reward, and replay combine to support the encoding of spatial experience. The presence of reward, especially unexpected reward encountered during learning, results in the release of dopamine from the ventral tegmental area throughout the brain, including in the hippocampus (Schultz et al., 1997). Although dopaminergic terminals in the hippocampus are sparse, many studies have shown strong physiological responses of hippocampal activity to dopamine. Dopamine in the hippocampus is known to facilitate synaptic plasticity by increasing the window in which spike timing dependent plasticity is induced (Brzosko et al., 2015; Zhang et al., 2009). Optogenetic activation of dopamine terminals in CA1 during experience results in increased recall of trajectories in a "crossword" maze, and enhances post-experience re-activation of place cells (McNamara et al., 2014). In this way reward can enhance synaptic plasticity involved in the formation of replays, as well as strengthening the representation of certain experiences through repeated replay of those experiences and even influencing future reward seeking behaviors.

Chapter 2: General Methods

Animal training and behavior

All animal procedures were compliant with the National Institutes of Health guidelines for animal use and approved by the JHU Animal Care and Use Committee. Experimental subjects were three to six month old male Long-Evans rats. Rats were food restricted to up to 85% of their stable weight during training and experimentation. Training occurred for roughly a week leading up to implant surgery. During training, rats were placed on one end of a linear track with chocolate drink (Nestle Breakfast Essentials) available at food wells on both ends of the track. Training sessions were terminated after either 30 minutes of exploration on the track or the completion of 20 full laps (out and back), whichever occurred first. A rat was considered well trained and ready for experimentation if he regularly completed 20 laps in less than 15 minutes.

After implant, rats completed three more training sessions on the experimental track (reward manipulation experiment, Chapter 3) or on the training track (V maze, Chapter 5). Rats were connected to wires in these sessions to allow them to adjust to the experiment conditions, but were otherwise similar to previous sessions. During experiments, position was monitored using headstage mounted LED lights and overhead cameras with 30 frames per second sampling rates.

Tracks were constructed from steel studs suspended 16 inches from the floor by upside down trashcans or wooden legs. The entire apparatus was spray painted black. Reward

wells were constructed from 5mL syringes with plastic piping attached to allow remote manual refilling of wells by the experimenter. All of these sessions occurred on a different track in a different orientation within the same room or in a different room from the track position used during the experiment. To prevent local olfactory cues between animals and sessions, tracks were cleaned with 75% ethanol after each use. However, stable visual landmarks were present such as the door of the experiment room, the location of the recording computer, and black tracks and pieces of cardboard which were taped to the walls in different locations and orientations to allow the rats to distinguish between walls.

Microdrive construction

Custom built microdrives holding forty individually moveable tetrodes were used for neural recording (Figure 2.1). Drive and cannula were designed using SolidWorks and printed by American Precision Prototyping with Polypro-like Accura 25 plastic material. Tetrodes were constructed from insulated 90% platinum/10% iridium wire from California Fine Wire, spun 80 clockwise turns and 40 counterclockwise turns per 10cm of tetrode and bonded by heating. Tetrodes were mounted to size 0-80 screws which allowed the tetrodes to be lowered 320 microns per one rotation of the screw. Prior to implant, the tetrode tips were plated with gold until impedance was no higher than 200-KOhms. For some experiments a stimulating electrode was included in the drive design (see Chapter 5 methods).

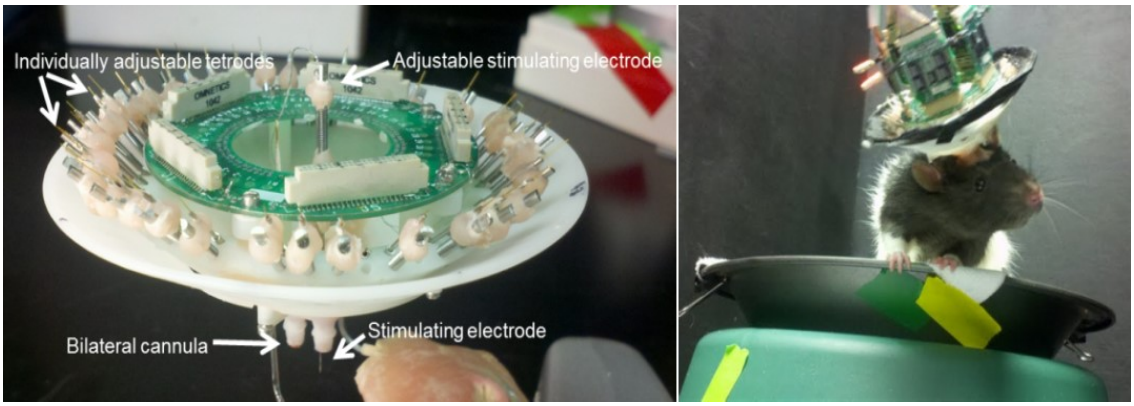

Figure 2.1. Microdrive design.

Forty tetrode microdrive with simulating electrode adaptation (left). Microdrive implanted on behaving rat (right).

Surgical procedure

All tools were autoclaved for sterilization within 24 hours before surgery. Rats were anesthetized with isoflurane and placed in ear bars on a stereotaxic stage. Isoflurane was maintained at 2.5-3.5% for the duration of surgery and surgery took place on a heating pad to prevent loss of body temperature under anesthesia. Eyes were coated with lubricant and covered with foil during surgery to prevent drying out. Prior to incision, the scalp was shaved and sterilized using betadine and alcohol prep pads and local anesthesia was induced with a subcutaneous injection of 0.2-mL lidocaine. A wide area of skull extending from several millimeters in front of bregma to behind lambda was exposed and cleaned of tissue. The nose position was adjusted if lambda and bregma were not level. Craniotomy locations were marked in pencil. For hippocampal recording

in area CA1, circular craniotomies of about 2-mm in diameter were located bilaterally at ±2.9-mm M/L and -4.2-mm A/P relative to bregma. Nine or ten bone screws, including one which was connected to a ground wire, were placed around the perimeter of the exposed skull, without drilling all the way through the skull and avoiding the suture lines. Then screws were covered with dental cement, leaving the location of the craniotomies clear. Craniotomies were performed by slowly drilling along the perimeter of the circle until the central piece of skull could be lifted out with forceps. Fine forceps were used to create an incision in the dura along the center of the craniotomy and then dura was peeled away. The microdrive was then stereotaxically lowered over the craniotomies until flush with the skull. The cannula was surrounded with bone wax and cemented in place. Then the scalp was sutured over the dental cement so that only a small region was left open around the drive cannula. Finally, the ground wire was connected to the drive using conductive epoxy, and all tetrodes were lowered 5 turns, or about 1.6-mm into the cortex. Rats were given buprenorphine at a dose of 0.01-0.05 mg/kg by intraperitoneal injection for analgesia during recovery. Post-surgical monitoring continued until rats became conscious about 20 minutes after being taken off isoflurane.

Tetrode adjusting and recording

Tetrode recording was performed using a Neuralynx Digital Lynx SX data acquisition system with Cheetah software, which allows simultaneous monitoring of local field potential and extracellular recording of single units. Local field potential was sampled at

3,255-Hz (rig 1) or 3,200-Hz (rig 2) and filtered at 0.1 to 500Hz. Four continuously sampled channels were available from each tetrode but for data storage purposes, only one channel was selected for recording. Spike triggered recording occurred when a threshold of 50-μV was exceeded, and sampled at 32,556-Hz (rig 1) or 32,000-Hz (rig 2).

In order to simultaneously move 40 tetrodes into the pyramidal layer of CA1, tetrodes were adjusted over two to three weeks while using local field potential as guidance. Adjusting is complicated by the large number of tetrodes which depress the brain tissue and swelling which is present for several days following surgery. During surgery the tetrodes were lowered into the cortex. This area is characterized by spiking activity that is less dense than in the hippocampal pyramidal cell layer, and spindles and delta waves are prominent during sleep. After tetrodes pass through the cortex, they enter the fiber rich corpus callosum where no spiking activity is observed. As the tetrodes reach the upper layers of CA1, sharp waves can begin to be seen as downwards deflections in the LFP. As the tetrodes move further down into the stratum oriens, ripples appear on the sharp waves as the waves increase in magnitude and then decrease as the ripples reach their maximum amplitude and spikes begin to appear. At this point, tetrodes should be moved no more than 40μm at a time to prevent moving past the pyramidal layer and damaging the cell bodies. Experiments begin when the experimenter decides that an optimal number of cells has been reached. It is often necessary to perform small adjustments at the end of experiment days due to continued movement of tetrodes. If a tetrode moves too far past the cell layer the ripple amplitude rapidly decreases while the sharp wave deflection reverses direction, and 8-Hz theta oscillations become stronger

during exploratory and active behaviors. Once a tetrode moves past the cell layer it can be moved back, but is unlikely to record as many cells as on the first pass due to tissue damage.

Analytical methods

Local field potential was analyzed from 4-7 tetrodes, located in the cell layer. Each raw trace was band-pass filtered in the 150-250-Hz ripple range and the smoothed Hilbert envelope (Gaussian kernel, 12.5-ms standard deviation) was averaged across all channels. Peaks in this signal exceeding 3 standard deviations above the mean were classified as SWRs (Figure 2.2). SWRs whose peaks were within 50-ms of each other were combined into one event. The boundary of the SWR is defined as the points at which the envelope passes the mean on either side of the peak. SWRs were used as candidate events for replay.

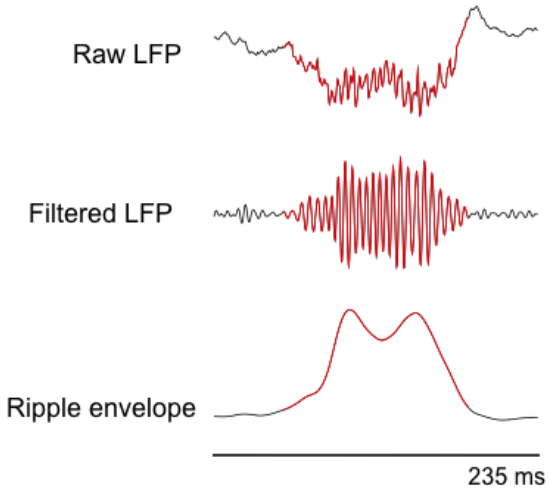

Figure 2.2. Example sharp wave ripple.

Raw LFP trace from a single tetrode channel (top). Single channel trace filtered between 150 and 250 Hz (middle). Average Hilbert envelope from 4 filtered LFP traces (bottom). Red indicates the boundaries of the SWR, defined as the time points in which the ripple envelope amplitude exceeds the mean.

Individual units were isolated by manual clustering of spike amplitudes using a custom cluster program (xclust2, Matt Wilson). Only units which were well isolated in four dimensional spike amplitude space and whose complex spike index (CSI) exceeded 5 were used in analysis. The CSI is a measure of the confidence that a unit is a pyramidal cell which takes into account both spikes occurring within the refractory period and the complex structure, or declining spike amplitudes within bursts (Quirk and Wilson, 1999). Place cells fire in their fields during locomotion so only the times when the rat was moving at a velocity of greater than 5-cm/s were used in analysis. Spikes were

binned into 1.8-cm position bins along the track and divided by the total time spent in each position bin to generate raw place fields. Smoothed fields were generated using a Gaussian kernel with a standard deviation of 3-cm.

Place field firing is dependent upon both position and heading direction and many cells re-map completely or rate re-map between directions on a linear track. For directional decoding, we calculated two fields for each unit, one for traversals from the bottom to top of the track and the other from traversals from top to bottom (Figure 2.3). Plotting the place fields in order of their field locations reveals the continuous population representation of the entire track by the place cell population. Plotting fields by the order of fields in the opposite direction reveals distinct codes for the two different direction trajectories (Figure 2.4).

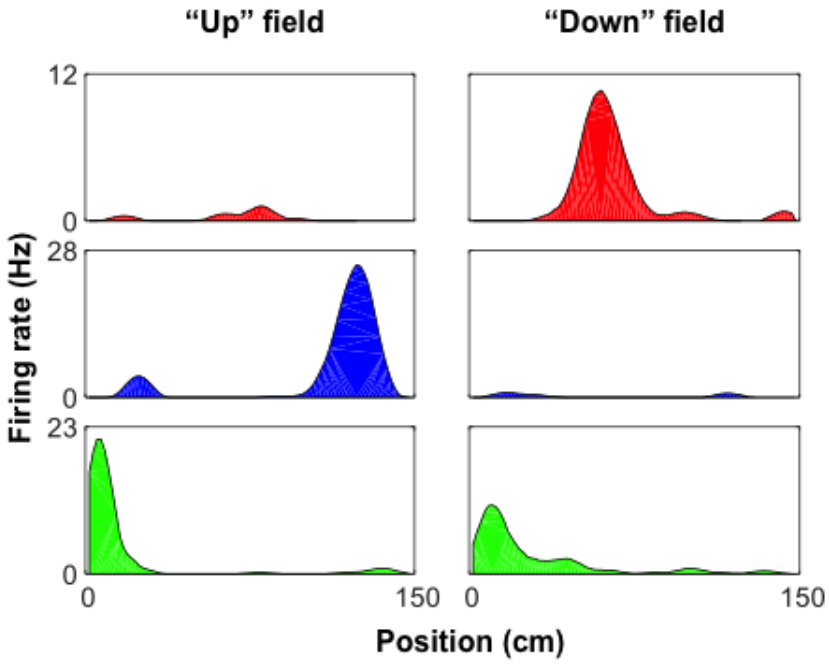

Figure 2.3. Example uni-directional place fields from three units.

A unit with a place field in the "down" direction (red), a unit with a place field in the "up" direction (blue), and a unit which rate re-maps between directions (green). Data from rat W18, 8/17/14.

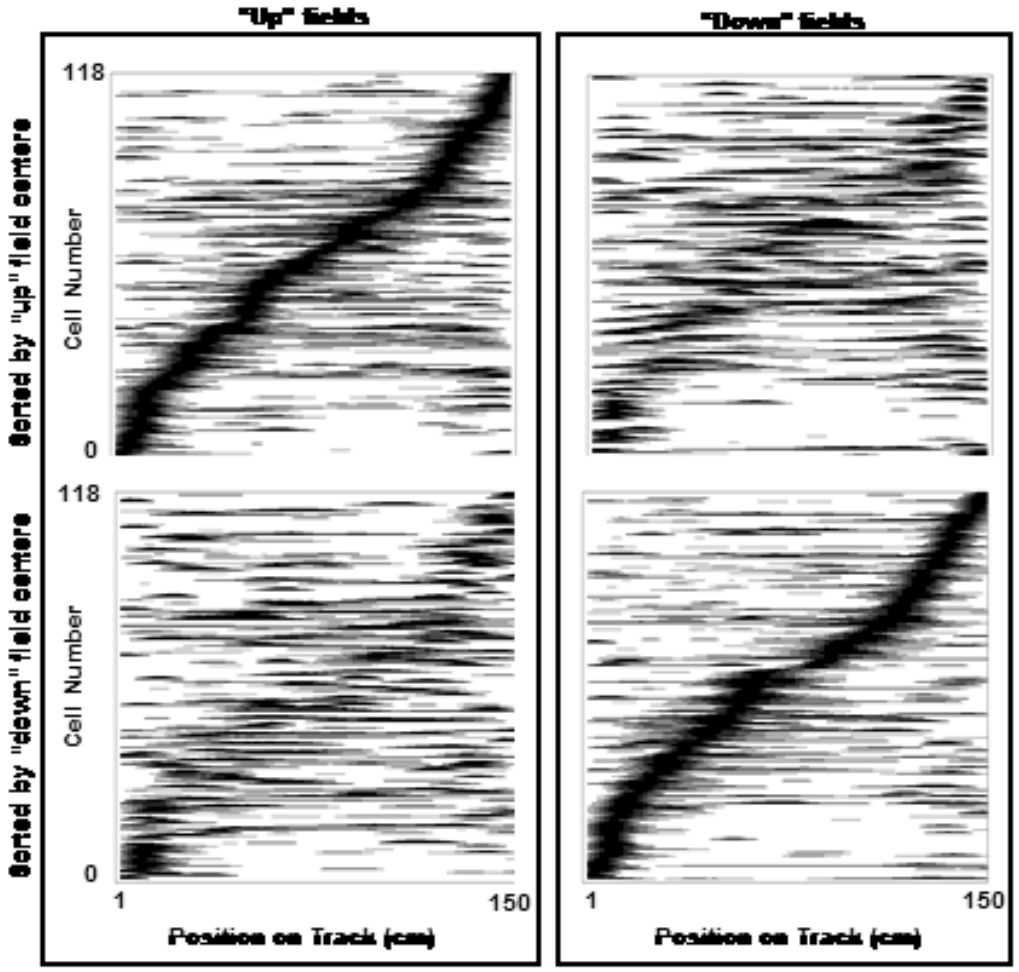

Figure 2.4. Uni-directional place fields.

Place fields from 118 simultaneously recorded units normalized to their peak firing rate, calculated from upwards runs (left) or downwards runs (right). Units are sorted on the y-axis by the location of the place field peak of the "up" fields (top) or by the "down" field peak location (bottom). Data from rat W18, 8/17/14.

Position reconstruction from place cell firing was accomplished using a Bayesian decoding method (Davidson, et al., 2009; Pfieffer and Foster, 2013). This method calculates the posterior probability of position on the track given that the probability of a cell spiking in a given position is described by a Poisson distribution with λ equal to the mean firing rate (place field height) of the neuron in that position. We assumed a uniform prior for position with the result that each time bin makes an independent estimate of position. This is a conservative approach to limit the spurious detection of sequences. The posterior probability was calculated using the following equation:

$$\Pr(pos \mid spikes) = C(\prod_{i=1}^{N} \tau f_i(pos)^{n_i}) e^{-\tau \sum_{i=1}^{N} f_i(pos)}$$

where $f_i(pos)$ is the firing rate of the i-th unit as a function of position (the place field), n_i is the number of spikes from the i-th unit, τ is the length of the time bin, and C is a normalization constant which ensures that the posterior probabilities within each position sum to one (Davidson, et al., 2009). This function could be adapted to decode for position and direction using uni-directional fields. In this case the posterior probability is normalized across all positions in both directions. For behavioral timescale position reconstruction, we used non-overlapping 200-ms time bins. Candidate replay events were decoded in 10-ms overlapping bins of 20-ms in duration. Example decoding of position and replays occurring in the same behavioral episode are shown in Figure 2.5.

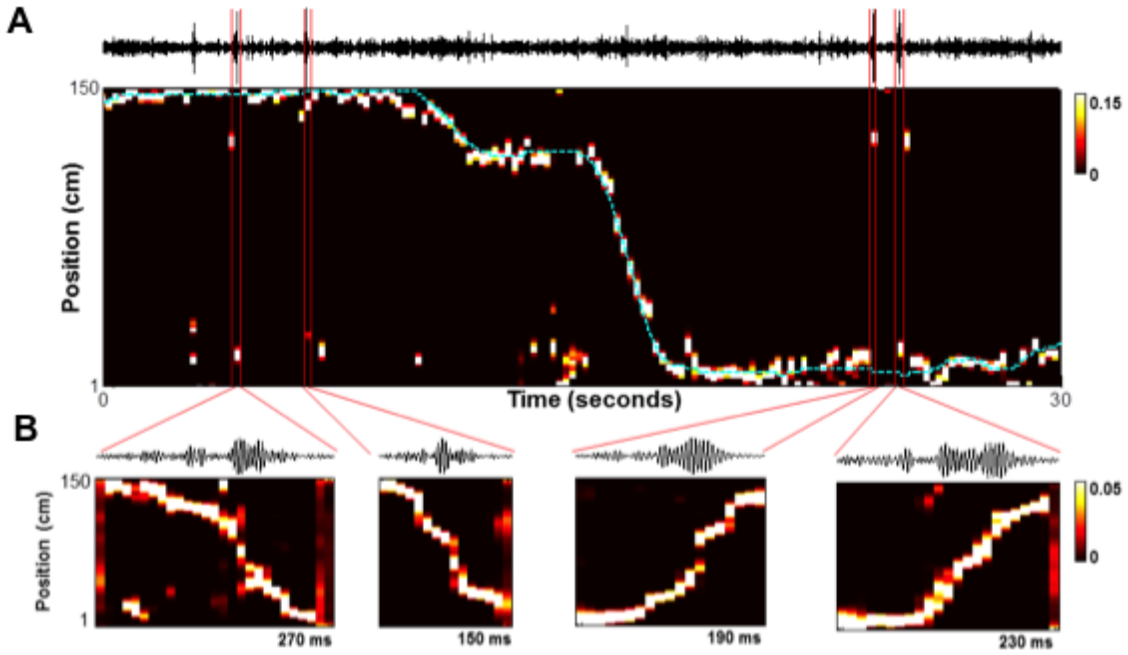

Figure 2.5. Example position and replay decoding.

(A) Ripple (150-250-Hz) filtered local field potential (top) and Bayesian decoding of the associated spike train during behavior (bottom). Actual position of rat overlaid in cyan. (B) Four example SWRs with associated replay that occurred at the ends of the track during the behavioral episode shown in A. Color scale indicates probability.

Replay events were confirmed if the candidate event met certain criteria. The weighted correlation of the posterior probability, an adaptation of Pearson's correlation, was the first criterion. Weighted correlation was calculated as follows:

$$Weighted\ Mean = m(x;w) = \sum_{i=1}^{M}\sum_{j=1}^{N} x_i w_{ij} \Big/ \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}$$

$$Weighted\ Covariance = cov(x, y; w)$$

$$= \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}\,(x_i - m(x; w))(y_j - m(y, w)) \Big/ \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}$$

$$Weighted\ Correlation = \ corr(x, y; w) = \ cov(x, y; w)/\sqrt{cov(x, x; w)cov(y, y; w)}$$

where x_i is the i-th time bin, y_j is the j-th position bin, w_{ij} is the probability at *(i,j)* and *M* and *N* are the total numbers of time and position bins, respectively (Wu and Foster, 2013). Candidate events with weighted correlations greater than or equal to 0.6 were classified as replays. Additional tests of replay significance are discussed in Chapter 3 methods. Directionality of replay was determined from the posterior probability of position and direction, which is also discussed in Chapter 3.

Chapter 3: Reverse replay of hippocampal place cells is uniquely modulated by changing reward

Background

Pyramidal cells in the CA1 region of the hippocampus, known as place cells, code for space by firing in a particular location in an environment called the place field (O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978), determined experimentally by correlating recorded spikes with position while an animal moves around. However, when the animal is at rest, place cells can be activated in temporally compressed population bursts that depict, on a faster timescale, behavioral trajectories through extended sequences of places. These events, often referred to as "replay", are associated with fast (150-250 Hz) oscillatory events in the local EEG known as sharp wave ripples (SWRs; (Buzsaki, 1986; Csicsvari et al., 1999).

SWR-associated replay was first reported in slow-wave sleep (Lee and Wilson, 2002), but later was found to occur also in the awake state whenever an animal pauses in an environment (Foster and Wilson, 2006). This first study reported one further, surprising property of awake replay – it played behavioral sequences in reverse, starting with the current location of the animal, and moving backwards along the preceding, incoming trajectory. In the study, animals happened to pause mainly at rewarded locations, so that these locations were where the reverse replay occurred. While this correspondence did not imply a relationship, it suggested a solution to the so-called "temporal credit

assignment problem" – the fact that an animal must make decisions about its movements when far away from a reward, and the reward by itself offers no perceptible guidance signal. In the classical place cell picture, there was no possibility of making direct associations between reward and cells whose place fields were far away, since when the animal is at the reward, these cells would be silent. Reverse replay at the reward offered a potential mechanism to associate place cells firing along the incoming route with estimates of future reward, and so was interpreted as a learning mechanism (Foster and Wilson, 2006).

Subsequently, reverse replay was replicated, but additionally it was found that forward replay also occurred during awake immobility (Csicsvari et al., 2007; Davidson et al., 2009; Diba and Buzsaki, 2007; Gupta et al., 2010; Karlsson and Frank, 2009). Although forward replay is not as theoretically attractive for temporal credit assignment as reverse, it does offer a more intuitive notion of memory recall. Thus, several recent studies have reported awake replay effects that were interpreted as forward planning of upcoming behavior (Jadhav et al., 2012; Pfeiffer and Foster, 2013; Singer et al., 2013). However, replay directionality was not directly measured in these studies. So the important question remains: do reverse and forward replay have different functions? In particular, does reverse replay have a specific relationship to reward, if it is involved in encoding recent paths to the reward? If so, is that relationship absent for forward replay, if forward replay is by contrast involved in recalling memories of how to get to reward when the animal is somewhere else?

To address these questions, we designed two experiments in which we varied reward magnitude, to determine the effect on hippocampal awake replays recorded during stopping periods. Previous studies that reported effects of reward and/or goal on SWRs or on replay have tended to utilize complex behaviors dependent on rather demanding learned tasks (Dupret et al., 2010; Pfeiffer and Foster, 2013; Singer and Frank, 2009). By contrast, we used running up and down the linear track, a task chosen for its simplicity, and its relative imperviousness to changes in reward. Animals were trained to run from one to the other, and did so regardless of whether the amount of reward was increased at one end, or even removed. Thus we could examine the effects of reward manipulation against a relatively constant behavioral background. In this classic design, in which forward and reverse replay were first reported, we hoped to uncover differences in the response of these two types of replay to increases or decreases in reward.

Methods

For general experimental methods, LFP and spike processing, and replay detection, see Chapter 2.

We verified our results with two additional replay detection criteria, increasing the weighted correlation threshold, or requiring candidate replays to pass a Monte Carlo shuffle. The Monte Carlo p-value was calculated as the number of shuffles of the posterior probability which passed threshold plus one divided by the total number of shuffles (1,500) plus one. Candidate replays with p-value less than 0.05 were confirmed

as replays. Both analyses produced very similar results to our original analysis (Table 3.2) although under the increased criteria we observed zero reverse replays at the removed reward which introduced a large amount of uncertainty to the linear model in experiment 2.

To determine a threshold for identifying directional replays we calculated the percent of the Bayesian posterior probability in the decoding from the "downward" fields:

$$downward\ probability\ = 100\% * \frac{\sum downward\ posterior}{\sum downward\ posterior + \sum upward\ posterior} \quad \text{(Equation 1)}$$

The distribution of downward probability has peaks close to 0% (strongly upward decoding) and 100% (strongly downward decoding) while the center of the distribution is roughly flat. The distribution from 33.5- 66.5% significantly deviated from the uniform distribution (Kruskal-Wallis, p=0.044) while the 34-66% region was not significantly different from uniform (p=0.080). Therefore, we classified all replays with downward probability greater than 66.5% as downward replays and forward probability greater than 66.5% as forward replays.

Generalized linear models

Generalized linear mixed effect models (GLMEMs) were used to analyze SWR and replay data. Since the data is in the form of rate, number of events per second the rat was at the reward well, the appropriate GLMEM is the Poisson family model with count as

the outcome and an offset term for time. This model accounts for random effects of subject, in this case "rat", and allows nesting of a second random effect, "experiment day", with the resulting sample size number of rats (5) rather than experiment days (7). Unique intercepts are allowed for each of the nested random effects while a single slope is estimated for the data set on the whole. This means that the uncertainties associated with the estimated mean rates, which are plotted as 95% confidence intervals in all figures, are not equivalent to the uncertainty of the difference between two conditions. This explains why the differences between some conditions are highly significant, even if their 95% confidence intervals are overlapping.

Our general model was used with different outcomes (SWR, replay, forward replays, and reverse replays) and with fixed effects based on the questions being asked. GLMEMs were fit using the glmer() function with family="poisson" from the lme4 package in R (freely available at http://cran.r-project.org). Multiple comparisons were corrected for using the default single-step method from the glht() function from the multcomp package in R. Model fits were assessed by visual inspection of residuals. Significance of coefficients was tested using Wald's z-test (asymptotic t-test) which is appropriate for Poisson distributed count data. Confidence intervals were calculated using the Wald method with similar results to those obtained from the profile likelihood method.

In the first main analysis (Figures 3.5, and 3.10-3.14), we used the categorical predictors of reward condition (unequal or equal) and end of track (unchanged or changed reward)

to estimate changes in rates of event (SWRs, replays, forward replays, and reverse replays) in both experiments. We calculated the difference between reward conditions and end of track relative to a reference condition, for example, the number of events per second that occurred in the equal reward condition and on the unchanged reward end of the track. The output of this model is summarized by:

$$Event\ rate\ =$$
$$\exp[b0\ +\ b1 * (unequal\ reward\ condition) +\ b2 * (changed\ reward\ end) +\ b3 *$$
$$(unequal\ reward\ condition * changed\ reward\ end)] \qquad \text{(Equation 2)}$$

The coefficient b_0 in a Poisson GLMEM is the log conditional mean event rate of the reference condition. By changing the reference conditions we were able to calculate the mean events per second under every condition. The coefficients b_1 and b_2 represent the log multiplicative change from the reference mean to the corresponding conditions. Thus, b_1 is the change from equal to unequal reward condition, holding end of track constant while b_2 is the change from unchanged reward to changed reward end of the track, holding reward condition constant. For interpretability, these coefficients were reported (in Table 3.2) and plotted with their 95% confidence intervals as the percent change in events/s between ends of the track:

$$Percent\ change\ =\ 100 * [exp(bi) - 1] \qquad \text{(Equation 3)}$$

The means and confidence intervals plotted in Figures 3.5C, 3.5F, 3.10C, 3.10F, 3.11C, 3.11F, 3.12C, 3.12F, 3.13C, 3.13F, 3.14C, and 3.14F come from the coefficient b_2. Meanwhile, the significance plotted in these figures comes from the interaction term, b_3, which is the difference between reward conditions of the difference in rate between ends of the track.

In later analyses we used categorical variables epoch, end of track, and replay directionality as fixed effects with the outcome variable replay. For the direct comparison of forward and reverse replays we extracted the forward to reverse difference for all epoch and end of track conditions, adjusting for multiple comparisons (Figure 3.16, Table 3.4). In the full comparison of increasing and decreasing phases we used all contrasts which differed by a single variable, adjusting for multiple comparisons (Figures 3.17B and 3.17D).

In Figures 3.6 and 3.15, we used epoch and end of track as fixed effects with the outcome variable SWR, replay, forward replay, or reverse replay rate.

$$Event\ rate\ =\ \exp[b0\ +\ b1 * (end\ of\ track) +\ b2 * (run) + b3 * (end\ of\ track * run)] \quad \text{(Equation 4)}$$

This allowed us to estimate conditional mean rates of each outcome variable at each epoch and end of track combination (Figures 3.6A and 3.15A). We could also extract all contrasts of interest from the model, adjusting for multiple comparisons (Figures 3.6B

and 3.15B). We estimated overall rates of SWRs and replays across runs by omitting end of track from the same model (Figure 3.7).

Additional analyses of running speed were performed (Figure 3.2). We analyzed this data using a linear mixed effect model which assumed normal distributions of running speed but was otherwise equivalent to the models discussed above.

Results

To test the effect of changing reward magnitude on SWRs and replay, we performed two experiments in which rats encountered varying reward while running on a linear track task (Figure 3.1). The experimental approach was similar for the two experiments, with the difference that experiment one tested the effect of increased reward and experiment two tested the effect of decreased reward. During each day of recording, rats were exposed to the same linear track in three successive epochs, during each of which rats completed 15-20 running laps (out and back) for liquid chocolate reward available at each end of the track. However, during epoch 2, the amount of reward available on each lap was changed at one end of the track only: in experiment one, the rewarded was 4X larger, while in experiment two, no reward was provided. Thus, epochs 1 and 3 tested baseline activity with equal reward available at both track ends, while epoch 2 tested the effect of a change in reward magnitude at one end of the track. Running performance was relatively impervious to the manipulation of reward, with modest differences in running speed for experiment two, but not for experiment one (Figure 3.2).

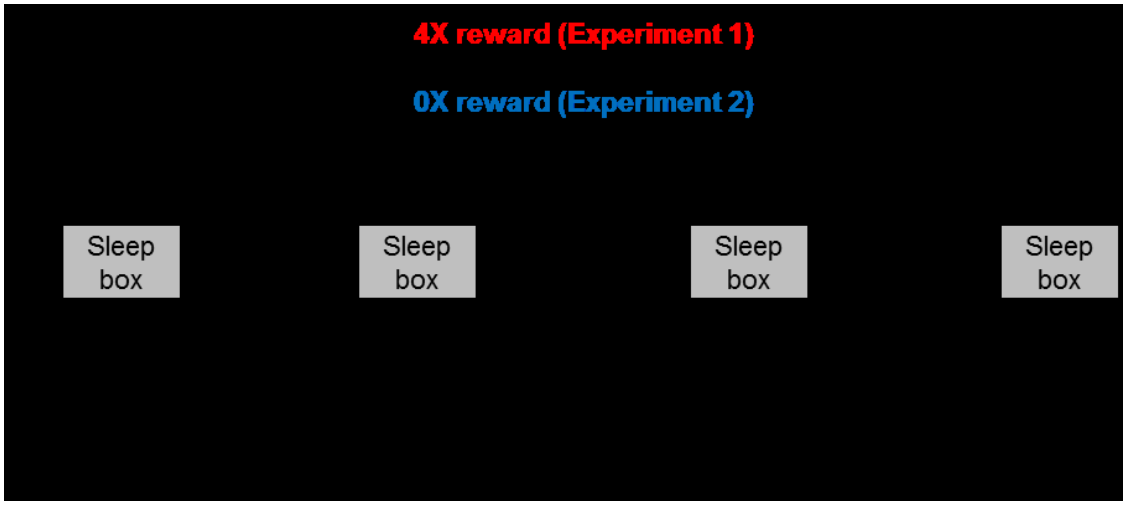

Figure 3.1. Experimental design.

Schematic of an experimental session. Reward was increased to 4X baseline at one end of the track in epoch 2 of experiment one, and decreased to 0X in experiment two.

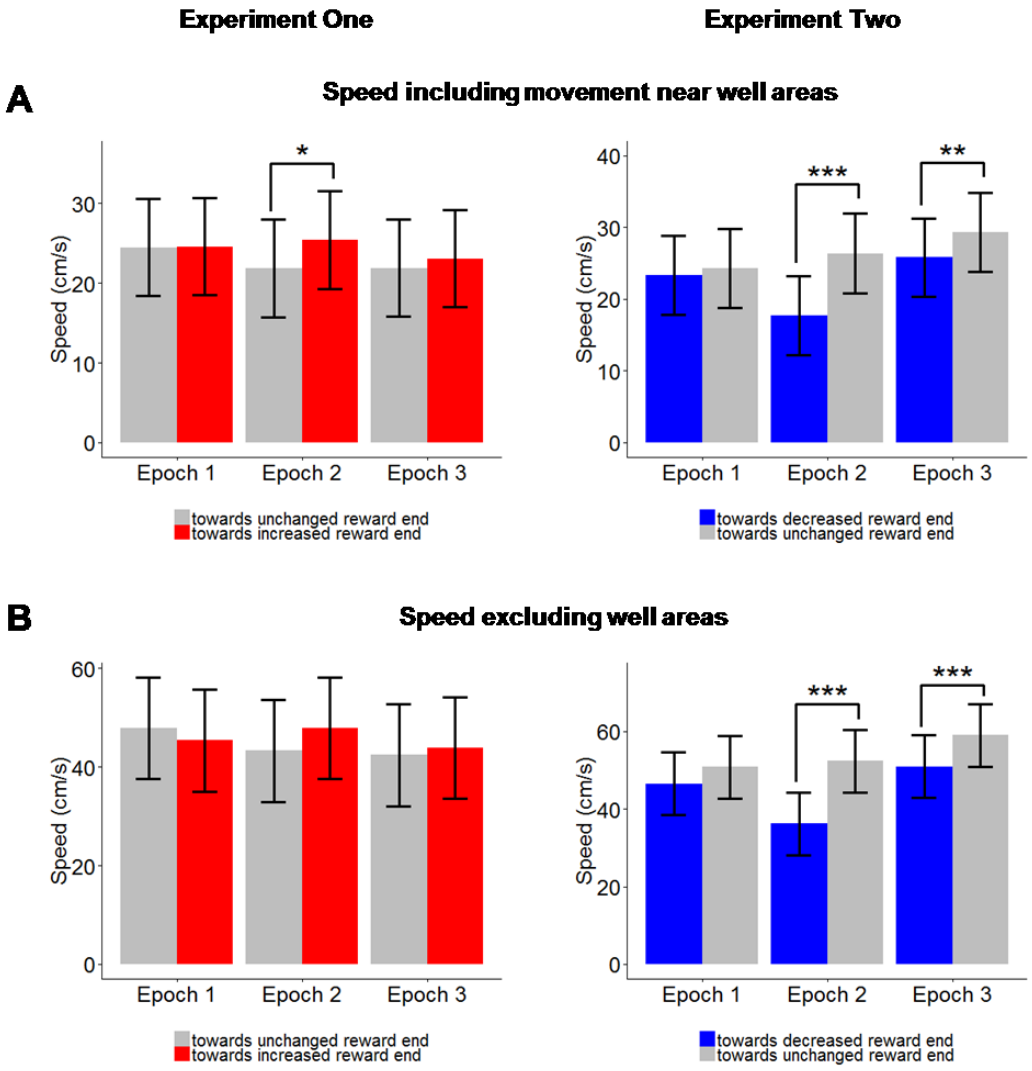

Figure 3.2. Mean running speed.

Mean running speed by epoch and direction on the linear track, with 95% confidence intervals as estimated by linear model (see methods). (A) Mean speed when speed exceeded 5 cm/s and the rat's location was further than 10 cm from the well locations. This criteria includes some slow movement and pauses at the end of the track preceding a run. (B) Mean speed when the rat was greater than 30 cm from the nearest well locations.

* indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

We tested five rats on both experiments for a total of 7 sessions of experiment one and 8 sessions of experiment two. In order to characterize the effect of reward changes on SWR events and on replay, rats were implanted with 40 tetrode microdrives targeting the CA1 region of the hippocampus, and single unit and EEG data were recorded concurrently. 120 ± 6 simultaneously recorded units were isolated per run. For each unit, we determined its place field on the track, and using Bayesian decoding methods on a coarse timescale (200ms), we were able to make very accurate estimates of the rat's actual position during running behavior (Figure 3.3A, Figures 3.4A and 3.4B). Then, during stopping periods at the track ends, we identified SWRs as peaks in ripple power (150-250Hz) in the EEG, and we applied Bayesian decoding on a fine timescale (10ms; see methods) to measure trajectory replay. On average, 15-20% of SWRs were determined to contain significant trajectory replay (Figure 3.3B, Table 3.1).

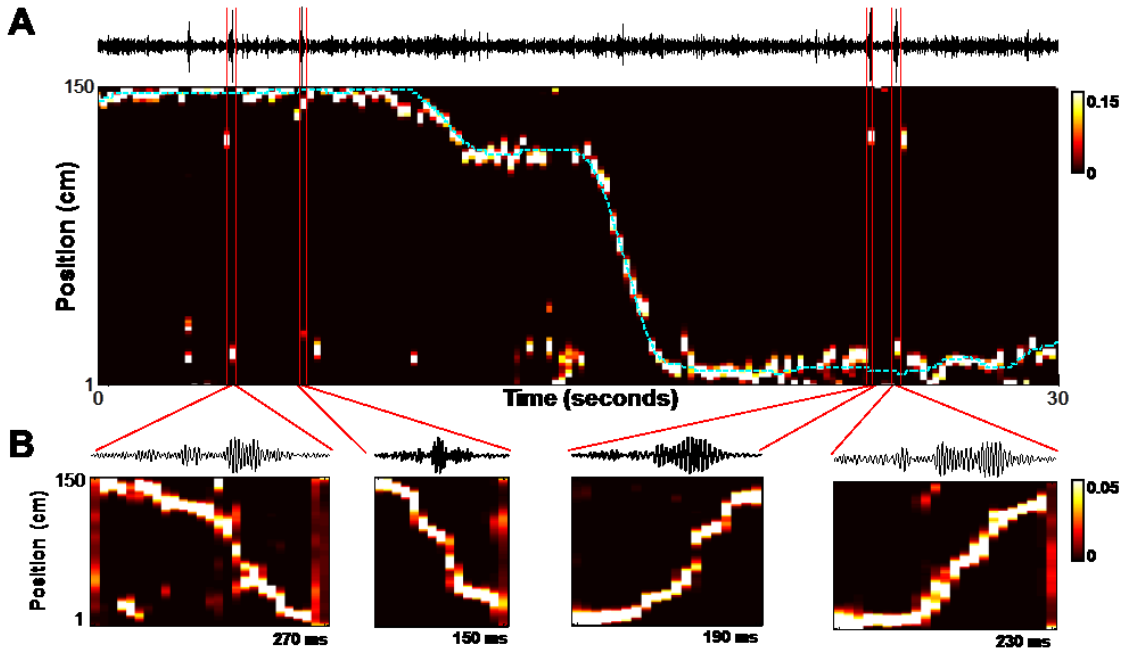

Figure 3.3. Place cell decoding

(A) Ripple (150-250Hz) filtered local field potential (top) and Bayesian decoding of the associated spike train during behavior (bottom). Actual position of rat overlaid in cyan. (B) Four example SWRs (top) with associated replay (bottom) which occurred at the ends of the track during the behavioral episode shown in B within the indicated time windows.

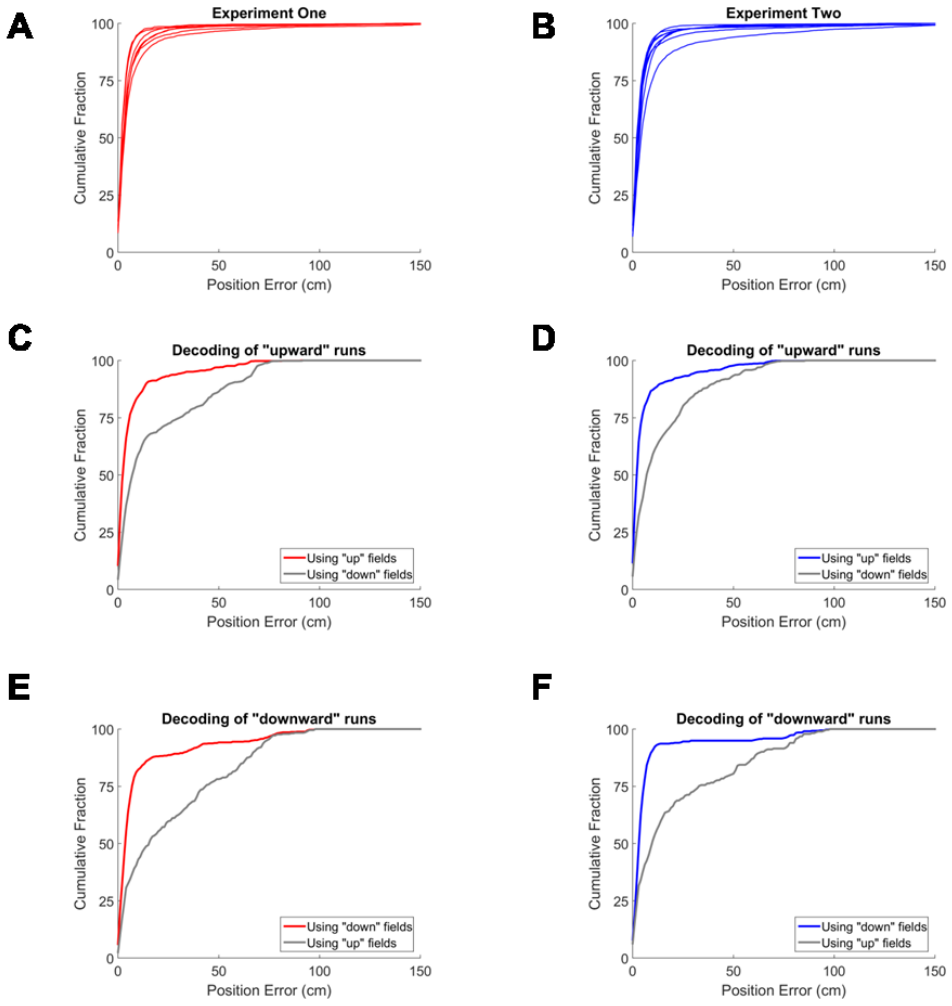

Figure 3.4. Cumulative error in position decoding

(A) Experiment one, error in position decoding during running using bidirectional fields. Each line represents a single experiment session. (B) Experiment two, error in position decoding during running using bidirectional fields. Each line represents a single experiment session. (C) Experiment one, group error in position decoding of "upward" runs, using directional fields. (D) Experiment two, group error in position decoding of "upward" runs, using directional fields. (E) Experiment one, group error in position decoding of "downward" runs. (F) Experiment two, group error in position decoding of "downward" runs.

Experiment	Rat	Day	Confirmed replays	SWRs
1	1	3	10	139
1	2	1	214	536
1	2	4	48	487
1	3	1	146	534
1	4	2	200	826
1	4	4	60	402
1	5	2	60	388
2	1	1	21	205
2	1	2	29	177
2	2	2	40	383
2	2	3	28	359
2	3	2	22	311
2	4	1	157	668
2	4	3	41	238
2	5	1	71	295

Table 3.1. Number of SWRs and replays detected on each experiment day.

SWR and replay frequency increases at increased reward

In the increased reward experiment (experiment one) SWR occurrence began to peak within seconds of the rat's arrival at the reward well area, before tapering off for the duration of the stopping period (Figure 3.5A). In the equal reward epochs the numbers of SWRs appeared similar at both ends of the track, however in the unequal reward epoch there appeared to be many more SWRs at the increased reward end. Although the rats stopped for significantly longer at the 4X reward than at the 1X reward (20.3 ± 2.8s and 8.1 ± 1.3s respectively, $p=0.031$, Wilcoxon signed rank test), the increase in SWR number was not simply due to a greater amount of time stopped but reflected an overall increase in the rate of SWRs (Figure 3.5B). Similarly to SWRs, the rate of replays increased at the increased reward end (Figures 3.5D and 3.5E).

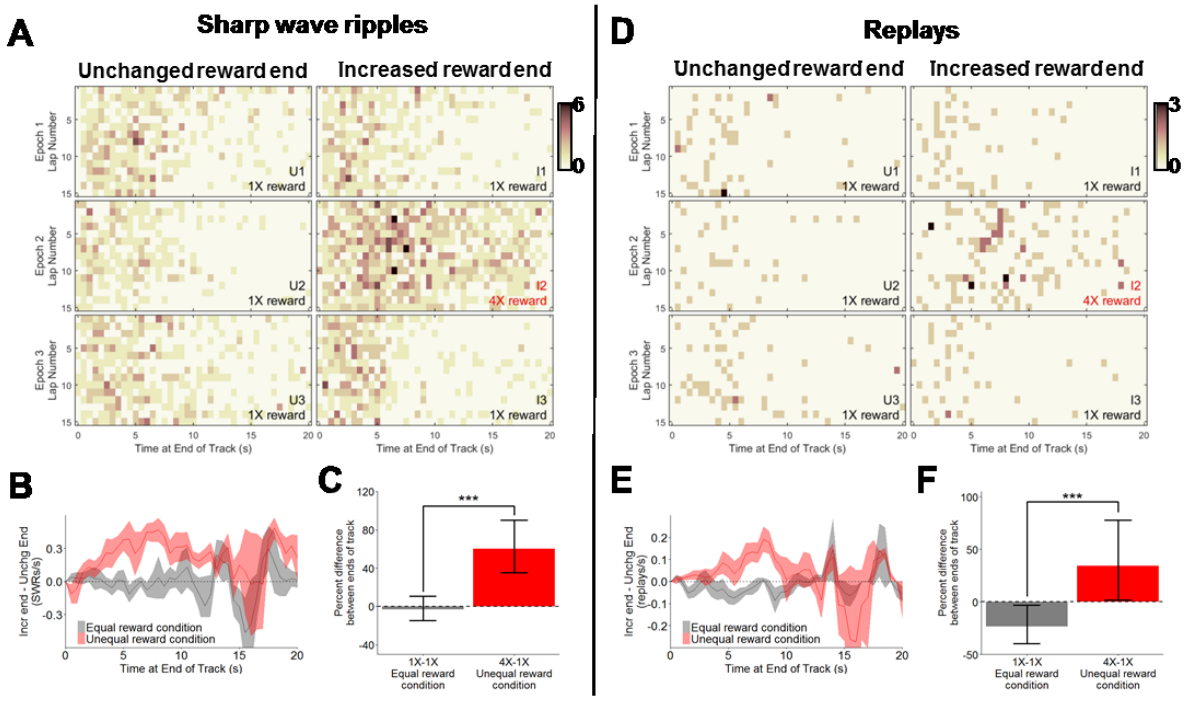

Figure 3.5. SWRs and replays are increased at 4X reward

(A) SWR occurrence during the first 20s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of SWRs. (B) Difference in SWR rate between ends of the track over the first 20s of each stopping period. Data shown as mean ± SEM. (n=maximum of 467 stopping periods in the equal reward condition and 217 in the unequal reward condition. Note that this number decreases over time due to variability of time spent at the reward well.) (C) Difference in SWR rate between increased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Replay occurrence, as shown in A. (E) Difference in replay rate between ends of the track over 20s, as shown in B. (F) Difference in replay rate between ends of the track, as shown in C. *** indicates $p < 0.001$.

To quantify these changes we applied a Poisson GLMEM to estimate the rate of events, defined as the number of events per second, at both ends of the track and under the two reward conditions (see experimental methods). The difference in SWR rate between ends of the track was much greater in the unequal reward condition (4X versus 1X reward) than when equal reward was present at both ends (Figure 3.5C, $z=5.24$, $p=1.61\times10^{-7}$). Similarly, the difference in replay rate between ends of the track was greater in the unequal reward condition (Figure 3.5F, $z=3.45$, $p=5.67\times10^{-4}$; Table 3.2). Thus, both SWR rate and replay rate reflected the increase in reward during epoch 2.

	Difference between:	0.6 threshold	0.7 threshold	0.6 threshold and shuffle
Replays	Reward conditions	76%	106%	88%
	Equal reward ends	-24%	-28%	-23%
	Unequal reward ends	34%	48%	44%
Forward Replays	Reward conditions	9%	34%	30%
	Equal reward ends	-11%	-17%	-1%
	Unequal reward ends	-3%	12%	30%
Reverse Replays	Reward conditions	88%	177%	182%
	Equal reward ends	-36%	-42%	-49%
	Unequal reward ends	56%	60%	44%
Forward Local	Reward conditions	-3%	21%	14%
Replays	Equal reward ends	-1%	-7%	15%
	Unequal reward ends	-4%	13%	31%
Reverse Local	Reward conditions	133%	163%	181%
Replays	Equal reward ends	-36%	-42%	-50%
	Unequal reward ends	50%	53%	41%

Table 3.2. Experiment one robustness analysis.

Comparison of reward condition analysis at different weighted correlation thresholds and shuffle requirement for replay detection. Red text denotes significance ($p<0.05$).

Interestingly, the difference in both SWR and replay rate between ends of track in the unequal reward condition was due to both an increase in rate at the changed end, and a decrease in rate at the unchanged end (Figure 3.6A, black squares – SWRs/s, black circles – replays/s). This suggests that the rate of events reflected relative rather than absolute reward magnitude. Thus, the overall rates of SWRs and replays were not sensitive to the increase in reward associated with epoch 2 (Figure 3.7A and 3.7B).

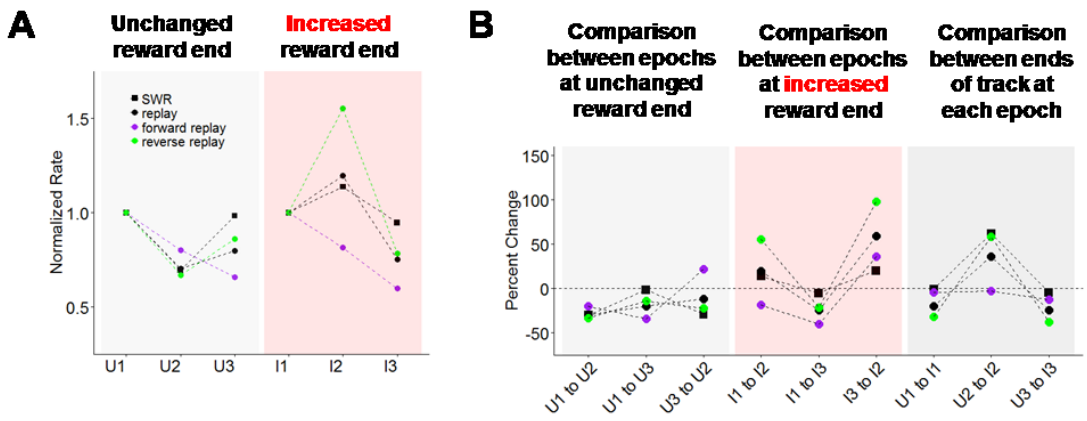

Figure 3.6. Experiment one changes by run and end of track.

Normalized rates (events/s) and between epoch and end of track changes in rates from Poisson GLMEM defined by Equations 3 and 4. (A) Normalized rate at ends of the track in experiment one. Data is normalized to epoch 1. (B) Changes in rate between epochs (left, center) and between ends of track (right) in experiment one. U-unchanged reward end, I-increased reward end. 1-epoch 1, 2-epoch 2, 3-epoch 3. Black square-SWRs, black circle-replays, purple circle-forward replays, green circle-reverse replays.

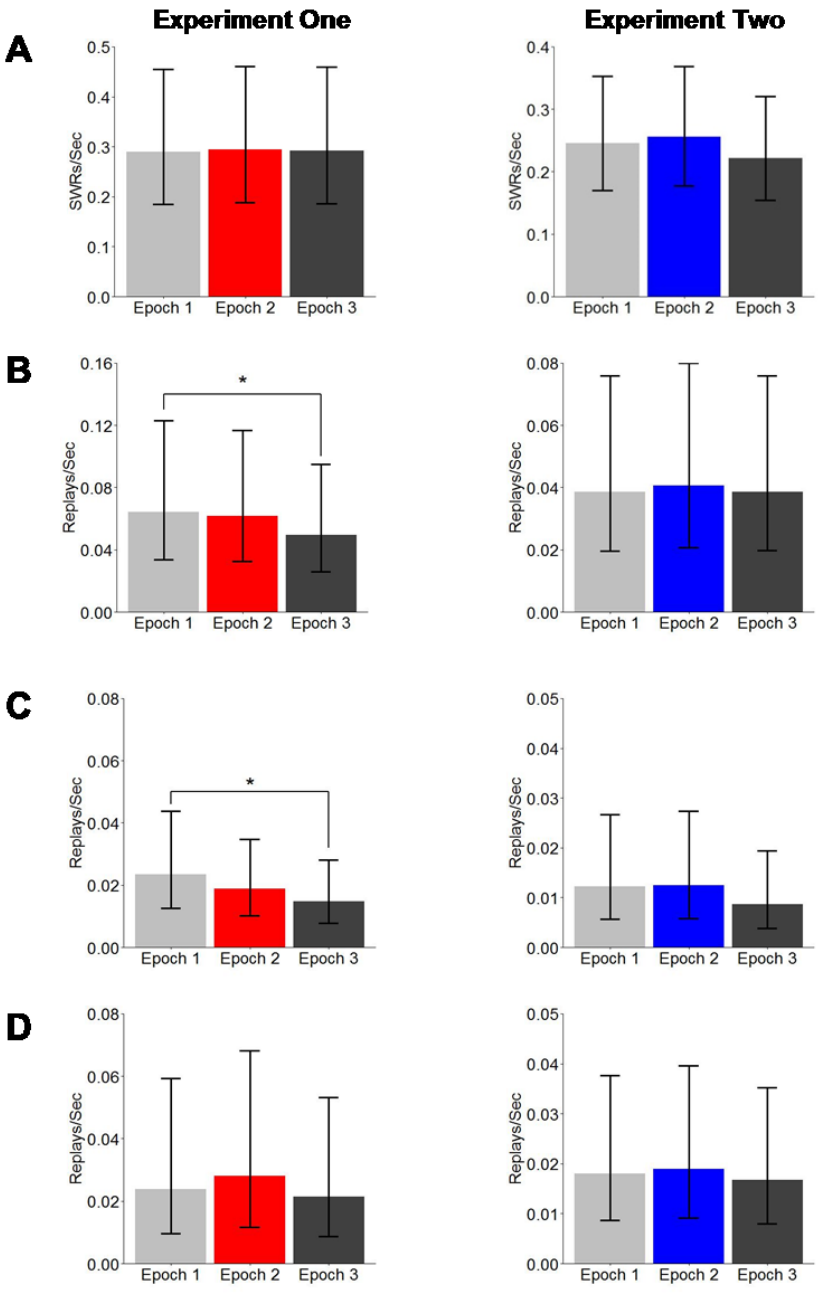

Figure 3.7. Overall rates across both ends of the track in both experiments.

Mean number of events per second in each epoch over both ends of the track, with 95% confidence intervals. (A) SWR rate. (B) Replay rate. (C) Forward replay rate. (D) Reverse replay rate. * indicates $p < 0.05$.

Differential behavior of forward and reverse replay at increased reward

Although we observed reward related changes in bi-directional replay rate, the contribution of forward and reverse replay remained unclear. We therefore classified the previously identified replays by their directionality, by calculating directional place fields for all units. Although some units had similar fields in both directions, the spatial representation of "up" and "down" heading trajectories were easily distinguishable (Figure 3.8A). Directional decoding accuracy was comparable to bi-directional decoding while control decoding of "up" runs using "down" fields and vis versa was poor (Figures 3.4C-3.4F). The original replays were re-decoded using directional place fields resulting in a posterior probability containing information on both position on track and heading direction (Figure 3.8B). Based on our criterion 79% (581/738 replays in experiment one) and 77% (314/409 replays in experiment two) of replays were classified as forward or reverse and the remaining 21% (157/738) and 23% (95/409) were omitted from further analysis. Thus, forward and reverse replays were non-overlapping subsets of the total replays that occurred concurrently during the experiment.

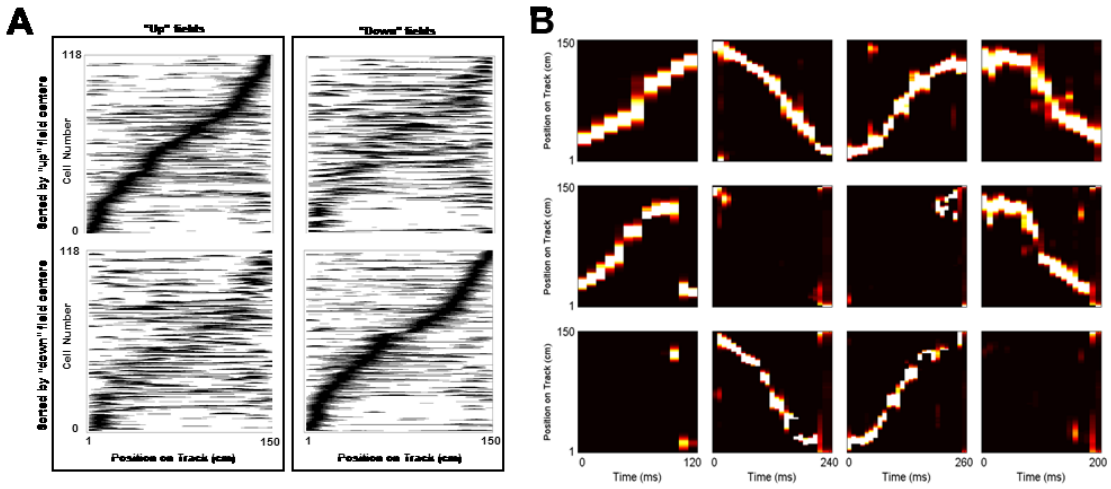

Figure 3.8. Directional replay decoding

(A) Unidirectional place fields of 118 simultaneously recorded units in CA1. "Up" direction (left) and "down" direction (right) fields, sorted by "up" field centers (top) or "down" field centers (bottom). (B) Four example replays decoded using bi-directional fields (top), "up" directional fields (middle) and "down" directional fields (bottom). Replays were assigned the following identities, from left to right: forward replay moving up the track, forward replay moving down the track, reverse replay moving up the track, and reverse replay moving down the track.

Critically, in order to evaluate the rate of forwards and reverse replay, it was necessary to observe both kinds of replay during the same stopping periods and independently of specific behaviors. It was previously reported on the linear track task that reverse replays occurred preferentially while rats faced away from the track at the end of the run, and forwards replays occurred preferentially after rats had turned around prior to

running the next lap (Diba and Buzsaki, 2007). However, in neither orientation was the relationship exclusive. We analyzed times when the rat was at the well location, defined as a 10cm radius around the well (Singer and Frank, 2009) during which time the rat's head direction was facing the well or within 90 degrees to the left or right for 83±1% of the time (mean±SEM). In our data, replays occurred in an interspersed manner relative to the beginning of each stopping period and to the beginning of each lap (Figure 3.9). Unlike Diba and Buzsaki, we did not observe a tendency of forward replays to occur in greater frequency before the next lap. We found no difference between forward and reverse replays in either the first or second half of the stopping periods (Wilcoxon rank sum test, first half/after lap: $p=0.56$, second half/before lap: $p=0.59$). Therefore, forward and reverse replay could be compared in our experiments independently during the same behavior.

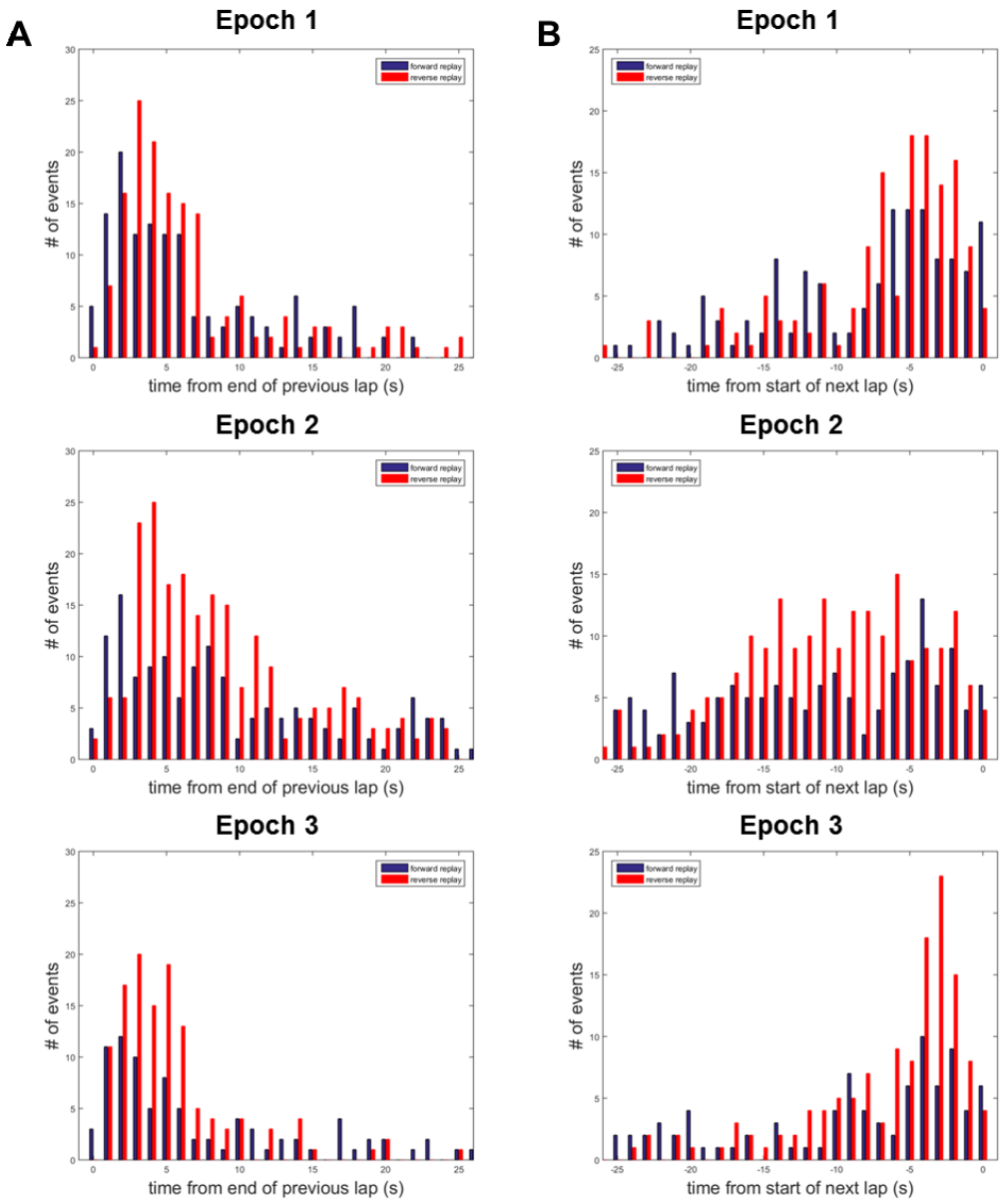

Figure 3.9. Time of forward and reverse replays within stopping periods.

(A) Time of replays aligned to beginning of stopping periods, and pooled over both experiments. (B) Time of replays aligned to end of stopping period and beginning of the next lap, and pooled over both experiments. The well area was slightly expanded to ensure that the beginning of the next lap would be correctly identified.

Forward replay rate was not affected by reward condition (Figure 3.10C, $z=0.305$, $p=0.76$; Table 3.2). The rate of forward replays was very similar on both ends of the track regardless of reward magnitude (Figures 3.10A and 3.10B). Reverse replays, on the other hand, showed a robust response to increased reward (Figure 3.10D). The difference between ends in the unequal reward condition was significantly greater than in the equal reward condition (Figures 3.10E and 3.10F, $z=3.57$, $p=3.60\text{x}10^{-4}$; Table 3.2). These data suggested that reverse replays are sensitive to the magnitude of reward, while forward replays are not. Further, reverse replay rates decreased at the unchanged end in epoch 2, thus matching the relative reward effect described above (Figure3.6, green circles; Figure 3.7D). This is not evident in the rate of forward replay (Figure 3.6, purple circles; Figure 3.7C).

As in previous studies, the majority of forward and reverse replays were locally initiated, that is, the replayed trajectory began at the rat's actual location (Davidson et al., 2009; Pfeiffer and Foster, 2013). Local replays comprised 86% of all replays (497/581) in experiment one and 78% of all replays (246/314) in experiment two. Our results held when considering only locally initiated forward and reverse replays (Figure 3.11; Table 3.2). Remote replays were too infrequent for conclusive analysis, averaging less than five forward or reverse replays per run.

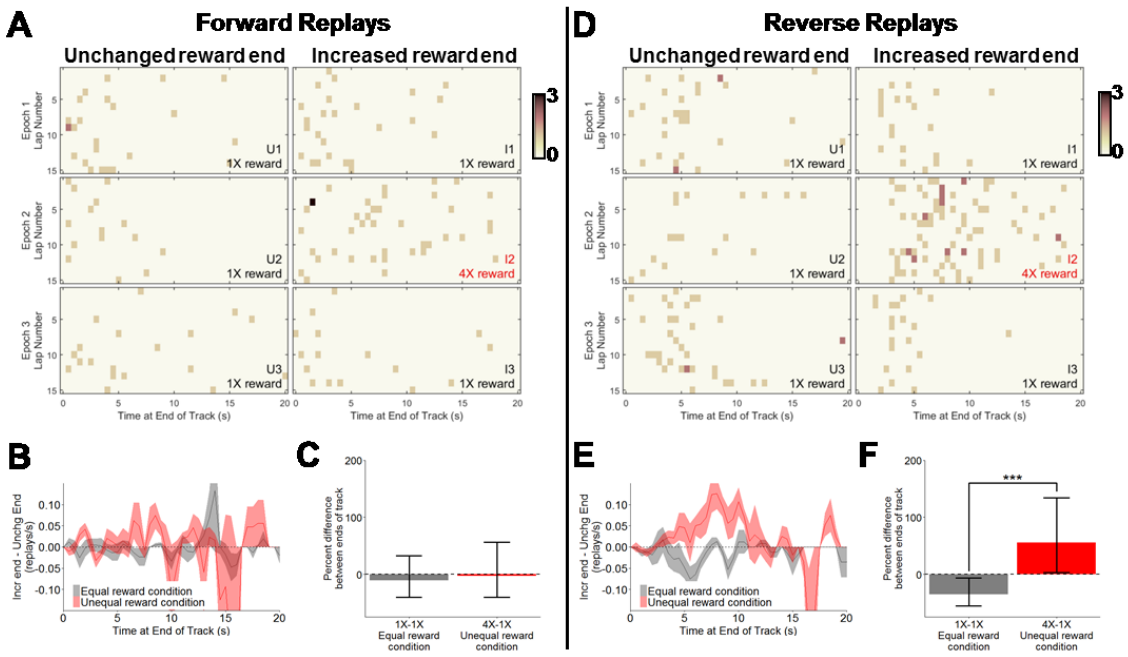

Figure 3.10. Reverse but not forward replays encode increase in reward.

(A) Forward replay occurrence in the first 20s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of replays. (B) Difference in forward replay rate between ends of the track in the first 20s of each stopping period. Data shown as mean ± SEM, as in Figure 2B. (C) Difference in forward replays rate between increased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Reverse replay occurrence as shown in C. (E) Difference in reverse replay rate between ends of the track over 20s, as shown in D. (F) Difference in reverse replay rate between ends of the track, as shown in E. *** indicates $p < 0.001$.

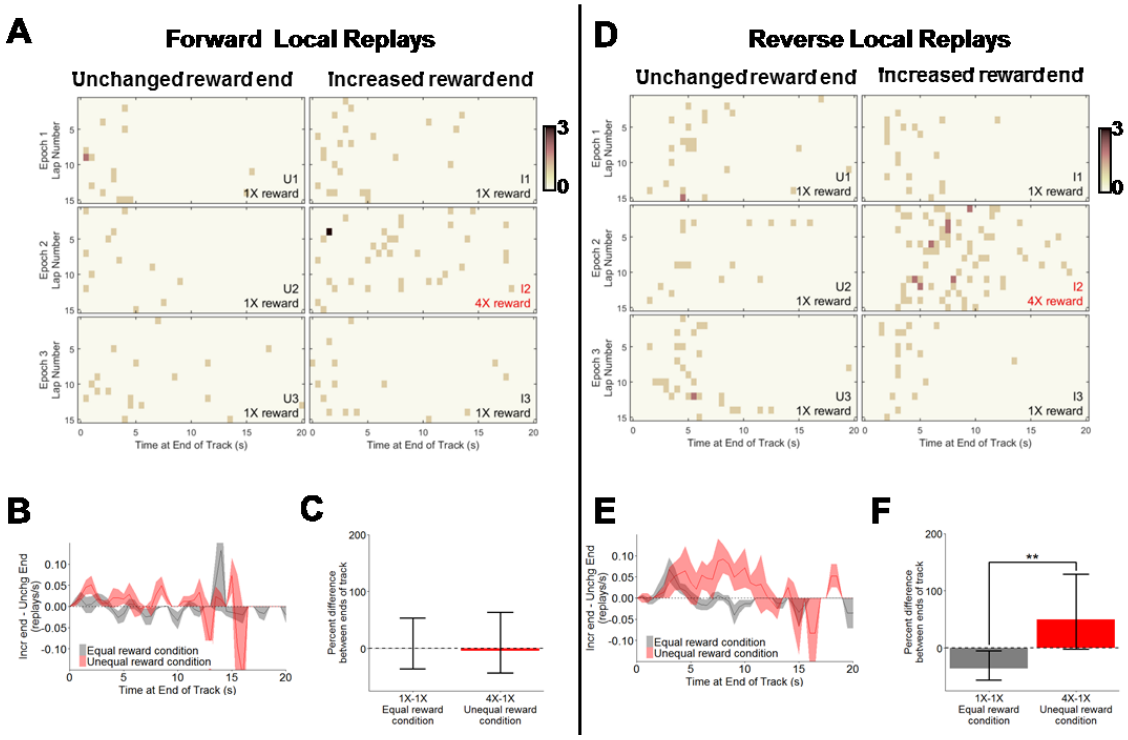

Figure 3.11. Locally initiated replays in experiment one.

(A) Local forward replay occurrence in the first 20 s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of replays. (B) Difference in local forward replay rate between ends of the track in the first 20 seconds of each stopping period. Data shown as mean ± SEM, as in Figure 2B. (C) Difference in local forward replay rate between increased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Occurrence of local reverse replays, as shown in A. (E) Difference in local reverse replay rate between ends of the track over 20s, as shown in B. (F) Difference in local reverse replay rate between ends of the track, as shown in C. ** indicates $p<0.01$.

SWRs and replays are decreased when reward is removed

In experiment two, reward was removed at one end of the track, to test the effect of a decrease in reward magnitude on replay. SWRs were dramatically decreased in the absence of reward (Figures 3.12A and 3.12B), and the difference between reward conditions was significant (Figure 3.12C, z=-6.66, p=2.67x10^{-11}). Replays followed a similar pattern to SWRs (Figures 3.12D and 3.12E), with a significant difference between reward conditions (Figure 3.12F, z=-3.16, p=0.0016; Table 3.3).

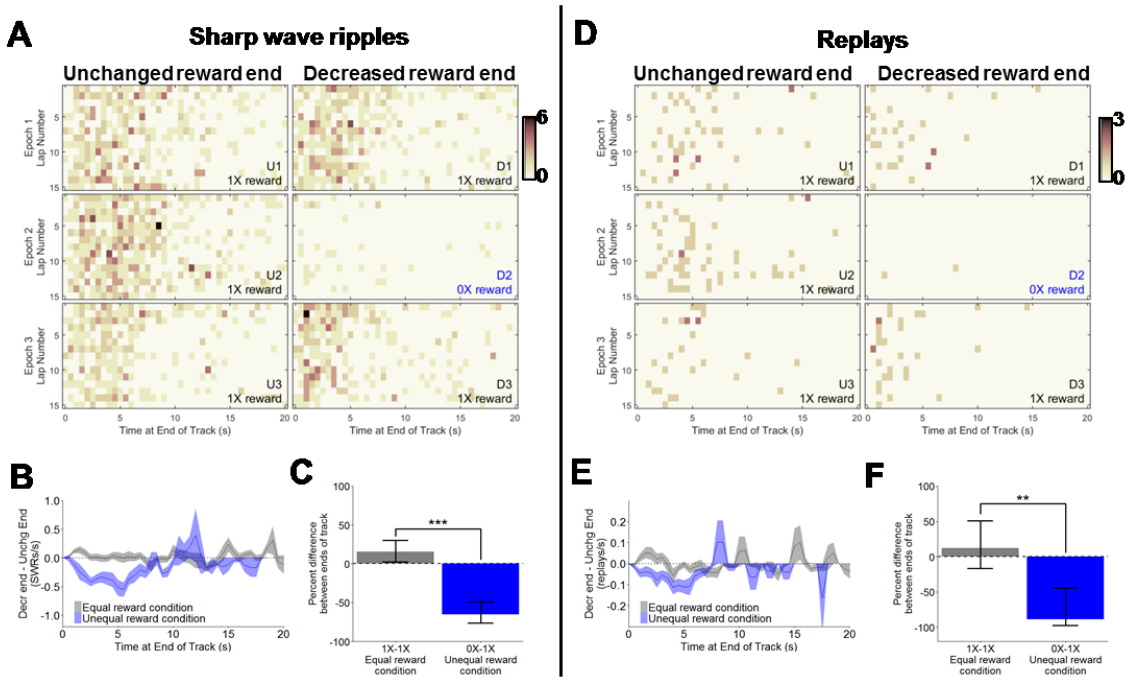

Figure 3.12. SWRs and replays are diminished in absence of reward.

(A) SWR occurrence in the first 20s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of SWRs. (B) Difference in SWR rate between

ends of the track in the first 20s of each stopping period. Data shown as mean ± SEM. (n=a maximum of 580 stopping periods in the equal reward condition and 230 in the unequal reward condition.) (C) Difference in SWR rate between decreased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Occurrence of replays as shown in A. (E) Difference in replay rate between ends of the track over 20s, as shown in B. (F) Difference in replay rate between ends of the track, as shown in C. ** indicates $p < 0.01$. *** indicates $p < 0.001$.

	Difference between:	0.6 threshold	0.7 threshold	0.6 threshold and shuffle
Replays	Reward conditions	-90%	-86%	-94%
	Equal reward ends	12%	17%	21%
	Unequal reward ends	-89%	-84%	-93%
Forward Replays	Reward conditions	-85%	-78%	-85%
	Equal reward ends	13%	11%	30%
	Unequal reward ends	-83%	-76%	-81%
Reverse Replays	Reward conditions	-88%	-83%	-100%
	Equal reward ends	-1%	0%	0%
	Unequal reward ends	-88%	-83%	-100%
Forward Local	Reward conditions	-82%	-76%	-84%
Replays	Equal reward ends	22%	9%	41%
	Unequal reward ends	-79%	-74%	-77%
Reverse Local	Reward conditions	-88%	-84%	-100%
Replays	Equal reward ends	15%	12%	11%
	Unequal reward ends	-86%	-82%	-100%

Table 3.3. Experiment 2 robustness analysis.

Comparison of reward condition analysis at different weighted correlation thresholds and shuffle requirement for replay detection. Red text denotes significance ($p<0.05$).

An important caveat in this experiment is that removal of reward elicited a shift in the rats' behavior. Instead of stopping to eat, the rats paused to scan and sniff the reward area for the missing reward. Overall, the rats spent less time stopped on the unrewarded end of the track (3.3 ± 0.5 seconds versus 8.5 ± 1.3 seconds at the rewarded end, p=0.0078, Wilcoxon signed rank test), although they continued to enter the unrewarded well area as required by the task. Due to these behavioral changes, very few forward and reverse replays occurred at the changed end in epoch 2 (Figures 3.13A and 3.13D). Nevertheless, our comparison of reward conditions, which is sensitive to the relative difference between track ends, again revealed a divergence between forward and reverse replay. There was no significant difference between reward conditions for forward replays (Figures 3.13B and 3.13C, z=-1.83, p=.068; Table 3.3), while there was a significant difference in reverse replays (Figures 3.13E and 3.13F, z=-2.05, p=.041; Table 3.3). These results were replicated by analysis of local replays only (Figure 14; Table 3.3). Thus, the sensitivity of replay to the relative rather than absolute magnitude of reward revealed an effect of reward removal on reverse replay, driven principally by increases in the rate of replay at the opposite end of the track, at which reward magnitude had not been changed but for which the reward magnitude was now relatively greater (Figure 3.15). The relative encoding of reward is further reflected by the stable overall rates of SWRs and replays across epochs (Figure 3.7).

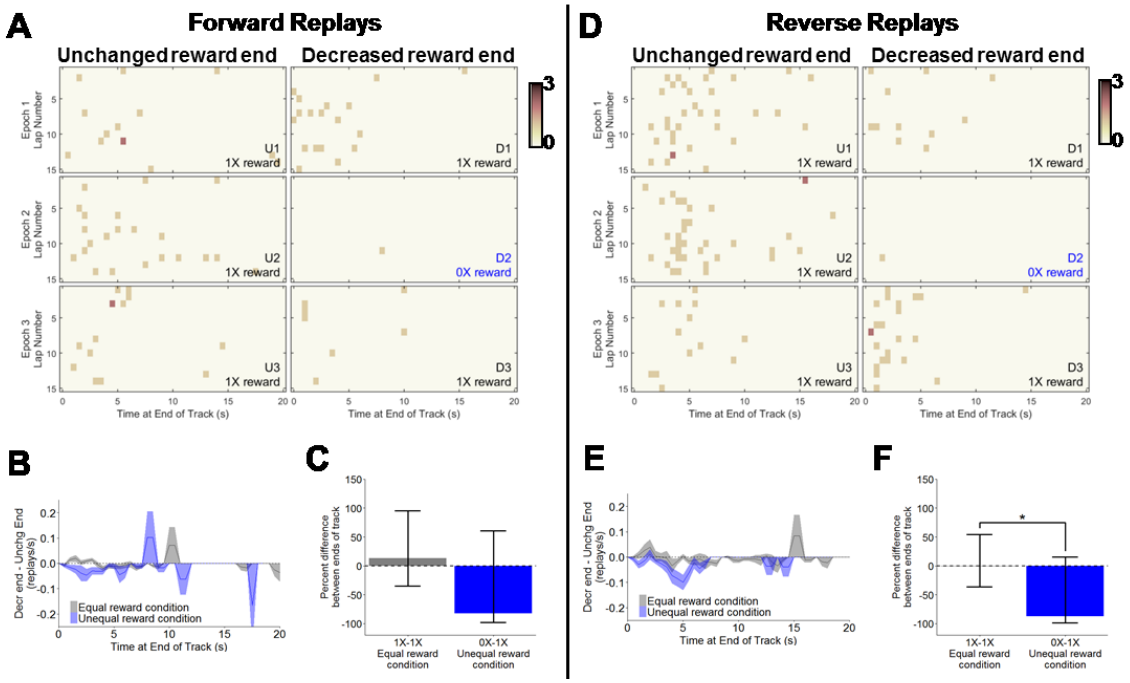

Figure 3.13. Forward and reverse replays at decreased reward.

(A) Forward replay occurrence in the first 20s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of replays. (B) Difference in forward replay rate between ends of the track in the first 20s of each stopping periods. Data shown as mean ± SEM, as in Figure 4C. (C) Difference in forward replay rate between decreased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Occurrence of reverse replays as shown in A. (E) Difference in reverse replay rate between ends of the track over 20s, as shown in B. (F) Difference in reverse replay rate between ends of the track, as shown in C. * indicates $p < 0.05$.

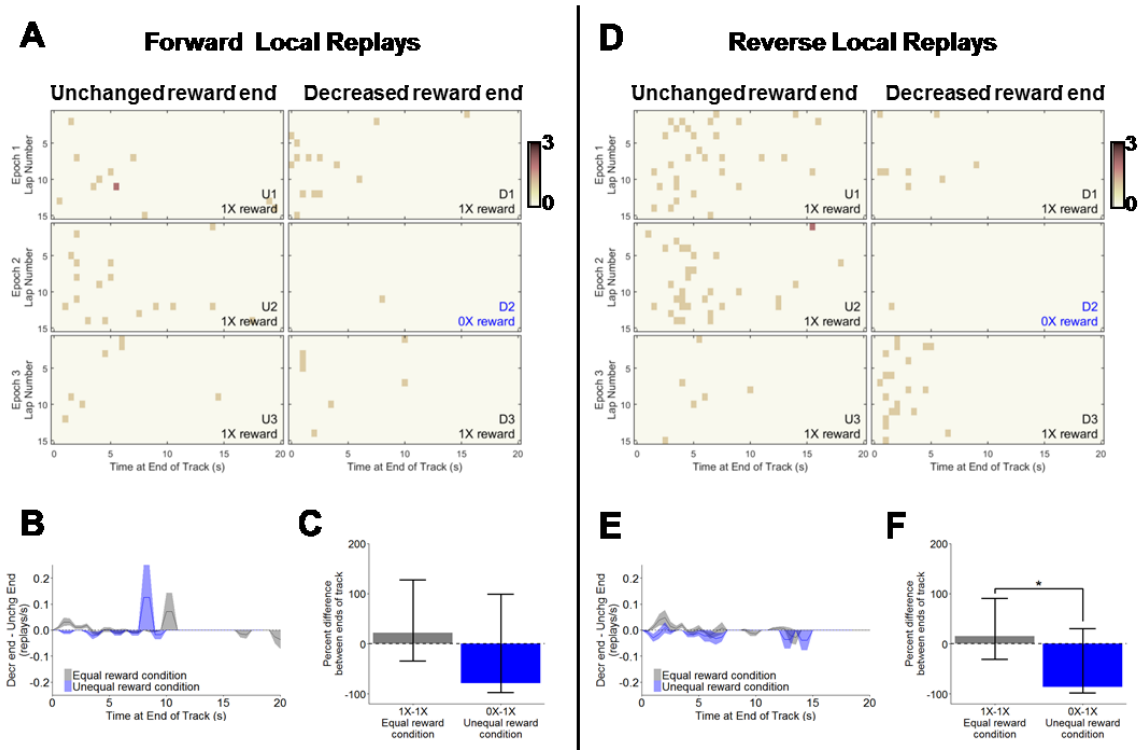

Figure 3.14. Locally initiated replays in experiment two.

(A) Local forward replay occurrence in the first 20s of each stopping period over 15 laps, summed across all sessions. Color bar indicates number of replays. (B) Difference in local forward replay rate between ends of the track in the first 20 seconds of each stopping period. Data shown as mean ± SEM as in Figure 4C. (C) Difference in local forward replay rate between increased and unchanged reward ends in the equal and unequal reward conditions, with 95% confidence intervals. (D) Local reverse replay occurrence, as shown in A. (E) Difference in local reverse replay rate between ends of the track over 20s, as shown in B. (F) Difference in local reverse replay rate between ends of the track, as shown in C. * indicates $p<0.05$.

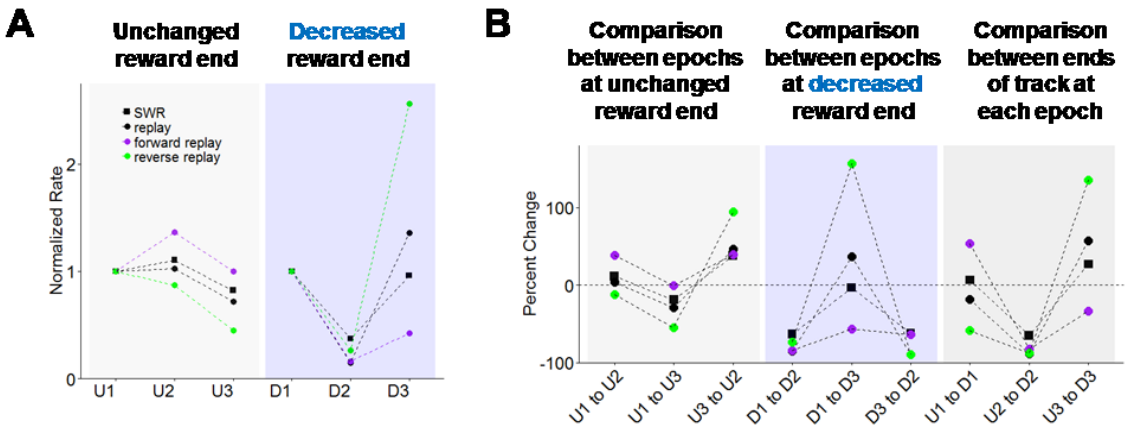

Figure 3.15. Experiment two changes by run and end of track.

Normalized rates (events/s) and between epoch and end of track changes in rates from Poisson GLMEM defined by Equations 3 and 4. (A) Normalized rate at ends of track in experiment two. Data normalized to epoch 1. (B) Changes in rate between epochs (left, center) and between ends of track (right) in experiment two. U-unchanged reward end, D-decreased reward end. 1-epoch 1, 2-epoch 2, 3-epoch 3. Black square-SWRs, black circle-replays, purple circle-forward replays, green circle-reverse replays.

Comparison of forward and reverse replays

While we have demonstrated through independent analyses that forward and reverse replay respond differently to reward changes, a direct comparison of forward and reverse replay in each condition would allow us to establish whether or not the two types of replay diverge under varying reward. We therefore constructed a Poisson GLMEM to estimate replay rate based on three variables: epoch, end of track, and replay

directionality. This more complex design is a more rigorous test of the data, with its implicit correction for multiple comparisons. Nevertheless, statistically significant differences between forward and reverse replay were observed in three conditions (Figure 3.16, Table 3.4). The weakest effect was an unexpected difference on one end of the track during epoch 1 of experiment 2 (Figure 3.16B, right). This track end did not correspond to a fixed physical location since location was randomized between sessions, but it may have corresponded to a predictive relationship between the end where the animal was initially placed at the beginning of epoch 1 and the end chosen to be manipulated in epoch 2. While both initial placement end and manipulated end were randomized between sessions, the pairing of the two variables was biased in experiment 2 but not in experiment 1, matching the result. Thus the unchanged reward end in experiment two frequently corresponded to the location where the rat encountered the first reward of the experiment session and was located opposite to the end where he was set down after a potentially stressful experience of being transferred on to the track. It is important therefore to note that absolute levels of forwards or reverse replay are difficult to relate to absolute reward levels, because of behavioral biases and because of the more general issue that animals may find places rewarding for a number of reasons in addition to the location of specific rewards introduced by the experimenter.

In contrast to these baseline effects, the experimental manipulation of reward magnitude during each experimental day was hypothesized to have specific effects on the ratio of forward and reverse replay, and these account for the two stronger effects identified by the GLMEM. First, the rate of reverse replays was significantly greater than the rate of

forward replays at the site of increased reward, in epoch 2 of experiment 1 (Figure 3.16A, $z=4.30$, $p=0.0001$). Second, the rate of reverse replays was significantly greater than the rate of forwards replays at the site of decreased reward, not on epoch 2 but on epoch 3, when the baseline reward level was reinstated (Figure 3.16B, $z=3.35$, $p=0.0049$). Interestingly, the reward level in this epoch was the same at both ends of the track, but the change in reward from epoch 2 to 3 corresponded to a large increase in reward, which may have accounted for the divergence between reverse and forward replay. Thus, this analysis identified a second form of relative response, sensitive to the change of reward magnitude with time.

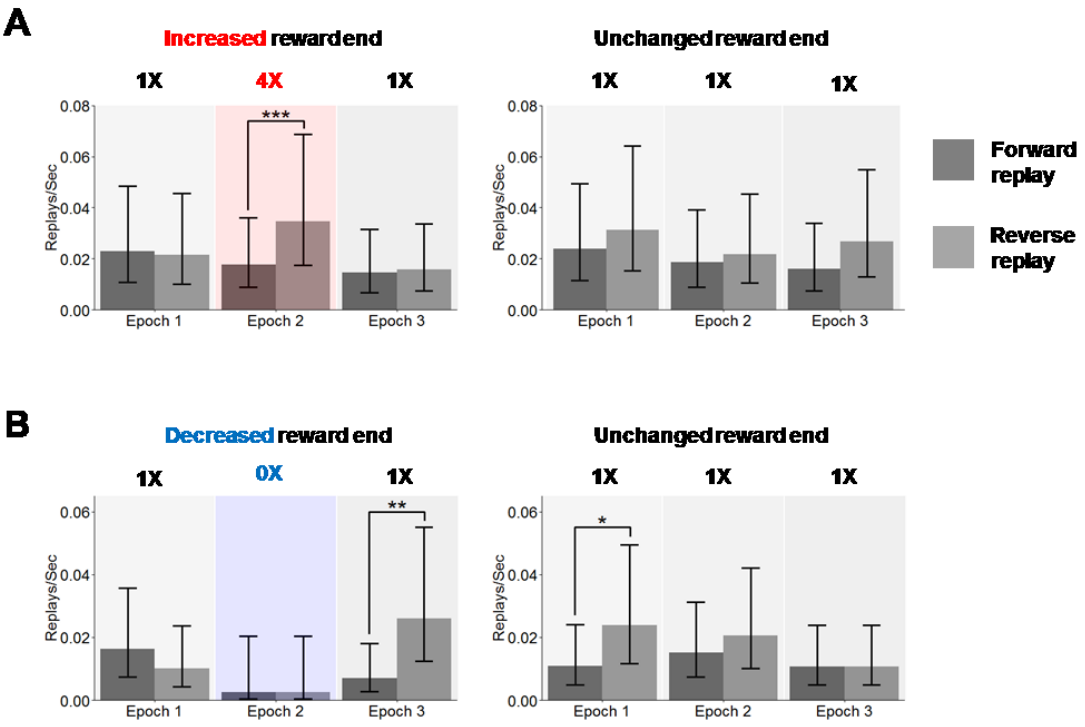

Figure 3.16. Comparison of forward and reverse replay

(A) Forward replay rate and reverse replay rate in epochs 1-3 in experiment one, with 95% confidence intervals. (B) Forward replay rate and reverse replay rate in epochs 1-3 in experiment two, with 95% confidence intervals. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$ adjusted for multiple comparisons, see Table 3.4.

Experiment 1

End	Percent Change	Lower CI	Upper CI	Estimate	Standard Error	Z-value	P-value
U1	31.70	-23.51	126.79	0.28	0.21	1.33	0.70
U2	17.65	-36.20	116.95	0.16	0.23	0.70	0.98
U3	67.86	-10.26	213.99	0.52	0.24	2.18	0.16
I1	-6.45	-52.50	84.22	-0.07	0.26	-0.26	1.00
I2	95.16	29.57	193.96	0.67	0.16	4.30	1.05E-04
I3	7.69	-47.30	120.05	74.00	0.27	0.27	1.00

Experiment 2

End	Percent Change	Lower CI	Upper CI	Estimate	Standard Error	Z-value	P-value
D1	-38.09	-75.38	55.63	-0.48	0.35	-1.37	0.68
D2	0.001	0.00	0.00	1.20E-05	1.41	0.00	1.00
D3	275.00	32.63	960.33	1.32	0.40	3.35	0.0049
U1	121.05	7.30	355.40	0.79	0.28	2.89	0.0231
U2	36.36	-18.88	129.23	0.31	0.20	1.57	0.52
U3	0.002	-57.19	133.63	2.30E-05	0.32	0.00	1.00

Table 3.4. Comparison of forward and reverse replay.

Percent difference between forward and reverse replay rates at changed or unchanged reward end of the track in epochs 1, 2, and 3 as shown in Figure 3.16. Red text denotes significance ($p<0.05$).

Changes in replays reflect relative changes in reward

We hypothesized that forward and reverse replays across both ends of the track might reflect not only the magnitude of reward present, but relative changes in reward with time. Both experiments have an increasing phase and a decreasing phase (Fig 3.17A). We sought to determine if the phases were related. We again used the three variable

GLMEM model, but instead of identifying significant effects, we considered the coefficients associated with different comparisons, and asked whether across the two experiments, those comparisons associated with relative reward increases produced similar coefficients. Thus for each experiment there were twelve coefficients describing changes in replay rate for the increasing and decreasing reward phases, which were adjusted for multiple comparisons. All twelve coefficients were plotted on a grid where each square represents the normalized change in replay rate from the condition on the x-coordinate to the condition on the y-coordinate (Figure 3.17B and 3.17D).

In order to test the similarity of the two experiments' increasing and decreasing phases, we identified hypothesized correspondences between the two experiments. For example, the coefficient describing the increase in reward from epoch 1 to epoch 2 at the changed end in experiment one corresponded to the coefficient describing the increase in reward from epoch 2 to epoch 3 at the changed end in experiment two. All the other comparisons reflected similar hypothesized correspondences. Given the vector of coefficients for each experiment, aligned to reflect the hypothesized correspondences, we calculated the similarity of the vectors as the sum of squared differences between all corresponding coefficients. This value was then used as a test statistic. We performed a bootstrap analysis in which the twelve coefficients were randomly shuffled 10,000 times, generating a null distribution of phase similarity test statistics. The data test statistic for the increasing phases was compared to the null distribution and was found to be smaller (i.e. coefficients were more related) than 99.5% of the shuffled data (one-sided test, $p=0.005$, Fig 3.17C). The data test statistic for the decreasing phases did not reach

significance (Figure 3.17E, one-sided test, p=0.114), in line with the general difficulty noted above of identifying robust decreases in replay rate as opposed to increases. Nevertheless, we report a robust effect of similar responses to relative increases in reward across both experiments.

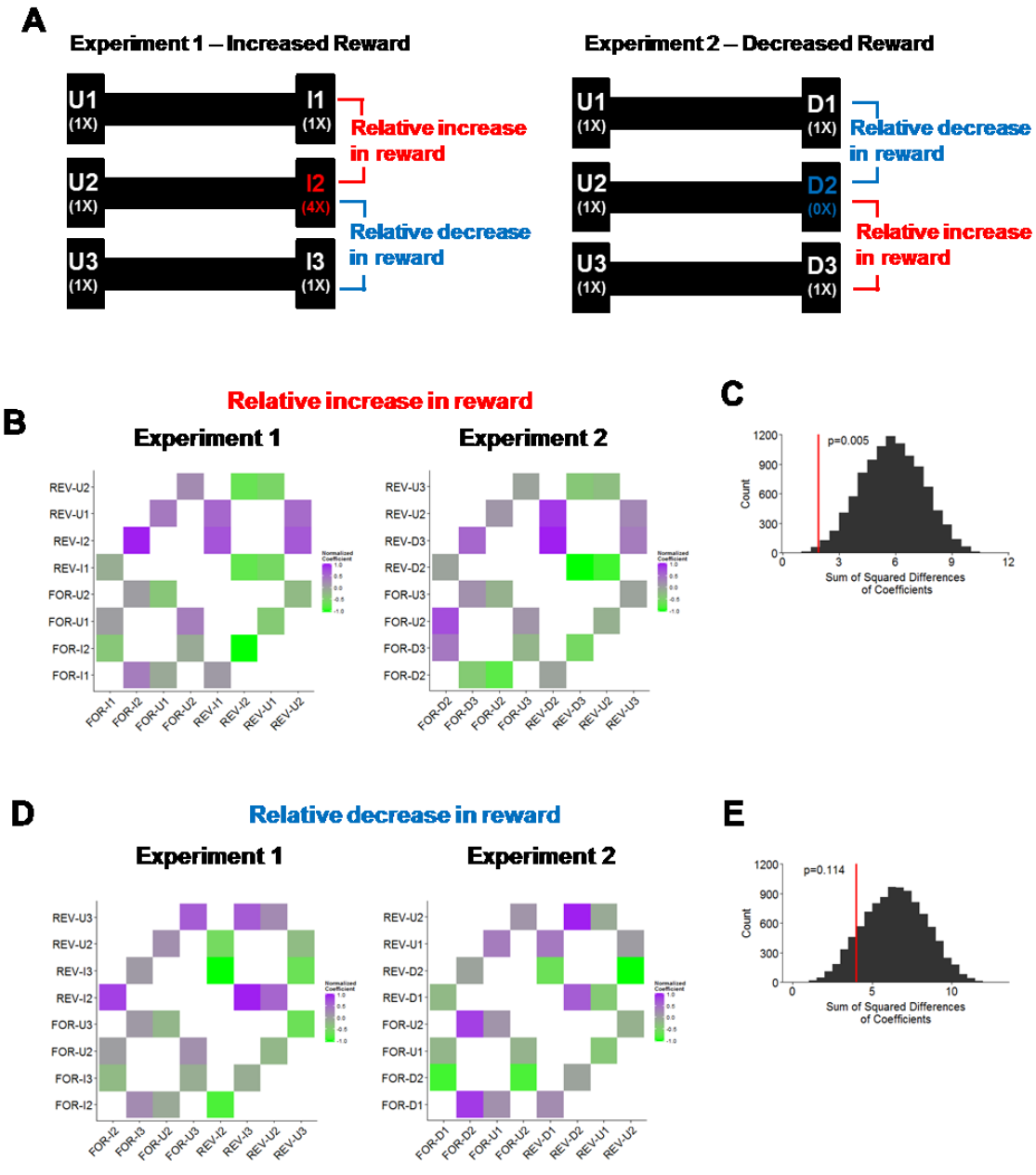

Figure 3.17. Changes in forward and reverse replay consistently code for relative increase or decrease in reward.

(A) Schematic of relative increasing and decreasing phases of experiments one and two.

(B) Set of coefficients from the linear model describing differences between forward and

reverse replays, ends of track, and epochs in the increasing phase of both experiments (experiment one, epoch 1 to epoch 2, and experiment two, epoch 2 to epoch 3). (C) Bootstrapped distribution of sum of squared differences between coefficients of experiments one and two, increasing phase. Red line represents data test statistic. (D) Set of coefficients from the linear model describing differences between forward and reverse replays, ends of track, and epochs in the decreasing phase of both experiments (experiment one, epoch 2 to epoch 3, and experiment two, epoch 1 to epoch 2). (E) Bootstrapped distribution of sum of squared differences between coefficients of experiments one and two, decreasing phase. Red line represents data test statistic.

In a complementary analysis, we calculated the significance of phase similarity test statistics between opposite decreasing and increasing phases (Figure 3.18). As all of these phases changed in opposite directions, we expected that these coefficients might be less related than chance. This would result in a data test statistic which is significantly greater than the mean of the null distribution. Indeed, for all four phase pairs, the data test statistics were larger than the majority of the null distribution test statistics and the difference between the decreasing phase of experiment one, and the increasing phase of experiment two reached significance (one-sided test, Figure 3.18D). Thus we found that reward changes of the same valence are similarly coded by forward and reverse replays, while the encoding of opposite valence changes appear to be anti-correlated, regardless of the absolute reward magnitudes involved.

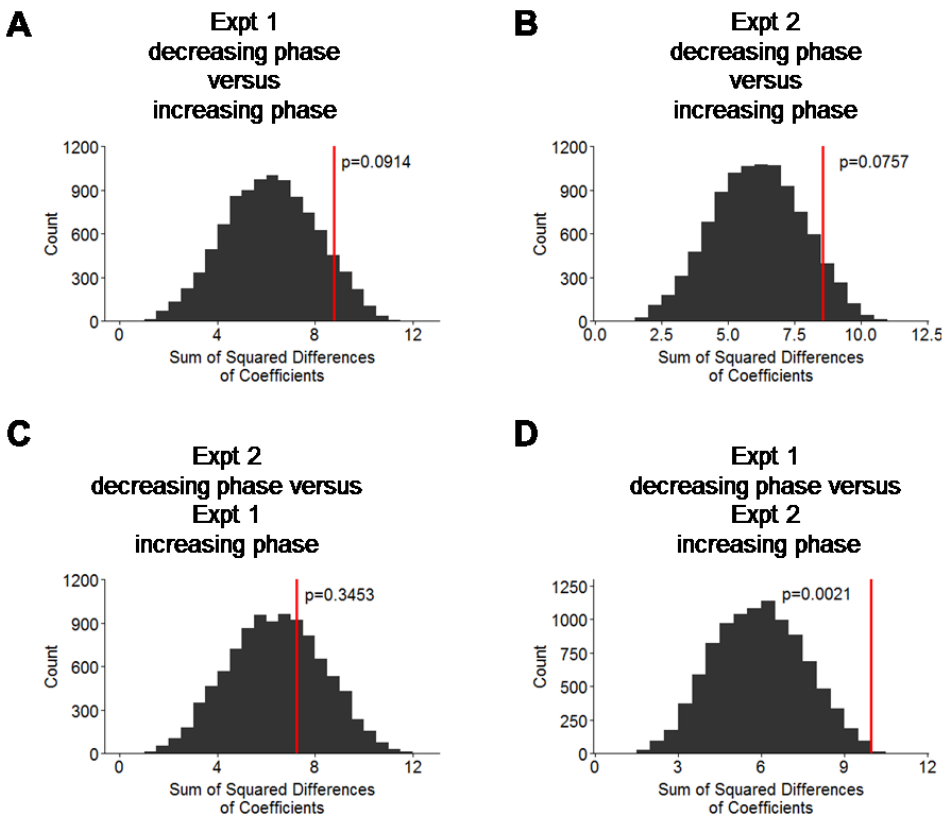

Figure 3.18. Comparison of replay rate coefficients between increasing and decreasing phases in experiments one and two.

(A) Bootstrapped distribution of sum of squared differences between coefficients in decreasing and increasing phases in experiment one. (B) Bootstrapped distribution of sum of squared differences between coefficients in decreasing and increasing phases in experiment two. (C) Bootstrapped distribution of sum of squared differences between coefficients in decreasing phase of experiment two and increasing phase in experiment one. (D) Bootstrapped distribution of sum of squared differences between coefficients in decreasing phase of experiment one and increasing phase of experiments two. Red line represents data test statistic.

Discussion

Reverse but not forward replays reflect changes in reward

We observed a striking difference between forward and reverse replay response to reward. Rates of reverse replay increased in response to increased reward, and decreased in response to decreased reward, while forward replays were unaffected. Moreover, reverse replays reflected relative reward amounts within an epoch, or the reward structure of the environment, as well as changes to reward between successive epochs. In experiment one, the increase in reverse replay rate at the 4X reward end of the track was accompanied by a decrease on the opposite end of the track, and reverse replays were significantly more abundant than forward at the increased reward (Figures 3.10 and 3.16). Even though the rats did not spend much time at the unrewarded location in epoch 2, we were still able to observe an increase in reverse (and not forward) replay rate with the restoration of reward at the changed reward end in epoch 3 (Figures 3.13 and 3.16). Overall, we found a consistent pattern of rate changes across both experiments that reflected effects of both relative magnitude between track ends and change over time for a given track end, across multiple instances (analysis of figure 3.17).

Our results relate to several earlier studies. Diba and Buzsaki first reported that both forwards and reverse replays occurred during stopping periods in a linear track running task (Diba and Buzsaki, 2007). They reported a tendency for reverse replays to occur while the animal was consuming reward with his back to the track, while forward replays were more common close to the onset of a new lap, presumably when the

animal faced towards the center of the track. Mindful of this, we restricted our analysis to times when the rat's head was within a 10 cm radius of the food well, which corresponded to the rat facing in the direction of the reward well over 80% of the time. Thus most of the detected replays occurred while the rat was facing towards the well in the consistent behavioral state of eating or preparing to eat and both forward and reverse replays occurred. This can be seen in Figures 3.10A and 3.10D, and Figures 3.13A and 3.13D in which replay occurrences are plotted on the same time axis. Furthermore, even under a less conservatively defined well area (30 cm around well), which included times in which the rat was facing towards the track and preparing to run, we found that significant numbers of both types of replays occurred throughout the stopping periods and were not clearly localized to the beginning or end of stopping periods (Figure 3.9). Therefore, our findings do not reflect a trivial behavioral change but rather the rebalancing of forward and reverse sequences during a period when both are *a priori* equally possible.

A pioneering study by Singer and Frank demonstrated that the presence of reward can lead to increased rates of SWRs, and showed an increase in coordinated re-activation of place cells during SWRs at reward compared to when reward is absent (Singer and Frank, 2009). We have extended these results by establishing that not only removal, but increases and decreases in reward are reflected by increases and decreases in SWR rate, respectively, and we explicitly demonstrate that only reverse replays respond similarly. This result is a compelling demonstration of the dichotomy between forward and reverse replay response to reward. Furthermore, SWRs are highly behaviorally

dependent (Buzsaki, 1986), and it is perhaps impossible to rule out subtle behavioral differences in the expression of different rates of SWRs, as opposed to neural circuit mechanisms. The same argument applies to the overall rates of detected replays. By contrast, observed forward and reverse replays rely on detection of similar spiking events, with only the temporal order reversed, so they act as controls for each other, and gross behavioral differences should be expected to impact them both equally (given the considerations discussed in the preceding paragraph). Additionally, in our experiments only 15-20% of SWRs were identified as replays. The remaining SWRs may contain remote replays of other environments (Karlsson and Frank, 2009) or may actually contain replays of the current environment if we were able to record from the entire CA1 population. We would not expect our random sampling of cells to favor one direction of replay over another so we can conclude that simultaneously recording from unlimited cells would yield similar results. Our findings suggest that it is reward driven changes in reverse replay rate that underlie the SWR effects reported by Singer and Frank. Moreover, our results strengthen the evidence for the involvement of the hippocampus in reward processing generally.

Reverse reactivation has also been shown to be stronger in the open field following high speed running periods (Csicsvari et al., 2007). In our experiments we observed running speeds which would be classified as high speed running on every lap, but faster running speeds leading up to larger reward did not explain our results. Although we observed faster speeds on laps towards the increased reward in experiment one, consistent with known effects of increasing reward (Crespi, 1942) , this effect was abolished when we

considered only behavior outside of reward well areas. This indicates that rats were not actually reaching higher velocities on laps towards the larger reward but rather taking more time to leave the increased reward well area after they finished eating. We observed lower running speed towards the 0X reward in epoch 2 of experiment two which also carried over into epoch 3, where reverse replays were increased in the opposite direction.

Implications for the functional role of reverse replay

Replay is implicated in hippocampal memory consolidation as well as the retrieval of memories to inform decision making (Carr et al., 2011; Girardeau et al., 2009; Jadhav et al., 2012) but whether replay directionality specifically contributes to these functions is unknown. Reverse replay enhancement in novel environments has been interpreted as evidence for its contribution to learning (Foster and Wilson, 2006). Unlike forward replays, the trajectory represented in a reverse replay is counterintuitive since rats do not run backwards down the track under normal circumstances. In nature, an animal may spend a large amount of time foraging for food and when food is found, the path that preceded its discovery becomes valuable. Locally initiated reverse replay may constitute a mechanism by which the representation of spatial experience preceding arrival at the current location can be mentally revisited and retroactively assigned value. Foster and Wilson proposed that the value assignment would be accomplished by pairing reverse replay with a reward triggered phasic dopamine signal (Foster and Wilson, 2006). It has recently been reported that hippocampal dopamine can transform

reverse order cell firing which normally results in LTD, into LTP at short delays consistent with the interspike intervals observed in replay (Brzosko et al., 2015). Although that study was performed at the CA3-CA1 synapse, while sequential connectivity may depend on the CA3 recurrent network, it suggests that under certain conditions reverse replay could actually strengthen forward associations. Also, increased SWRs at novel goal locations is predictive of future memory performance (Dupret et al., 2010). This indicates a functional significance of SWRs and presumably reverse replay in consolidation of reward related memory. Since most of our replays were locally initiated, the specific trajectory that was increased in abundance at the relatively larger reward was the reverse sequence representing the most recent trajectory the rat had taken. Thus, reverse replays may serve to strengthen valuable memories and contribute to consolidation.

Decreasing reverse replays at decreased reward suggests that when reward is removed, consolidation would be decreased. However, knowledge of a location which is no longer rewarded is also valuable information to remember so as not to expend energy visiting the unrewarded location. We observe enhancement of reverse replay rates at increased reward, while rates at the unchanged reward are decreased, an example of adaptive coding in the total rate of replays to represent the relative reward magnitude present. This could support the representation of relatively better options to facilitate the avoidance of lesser rewards while not explicitly encoding lack of reward.

Reverse replay coding of relative reward requires information about relative reward to be transmitted to the hippocampus. Interestingly, dopamine neurons of the ventral tegmental area display the same dynamical adaptation of neural output to the current range of reward magnitude as we observed in reverse replay (Tobler et al., 2005). Moreover, these neurons classically signal reward prediction error (Schultz et al., 1997) and this is consistent with our finding of rate changes associated with changing reward across runs, such as above-baseline rates after reinstatement of reward in experiment two. Ventral tegmental area projections may directly drive the adaptive coding response in the hippocampus by interaction with reverse replay representing ensembles. The latter possibility is consistent with recent reports that activation of dopamine neurons during experience can promote later hippocampal reactivation during rest and memory consolidation (McNamara et al., 2014). However, that study did not reveal whether reactivation during behavior was affected or if reactivation was related primarily to reverse or forward replay.

Implications for the functional role of forward replay

If reverse replay serves as a learning mechanism, forward replay is a likely candidate for memory retrieval and planning future paths (Diba and Buzsaki, 2007; Foster and Wilson, 2006). Planning is arguably simple or even unnecessary in our linear track task. The planning requirement in our task was not affected by the experimental changes to reward, since the required future path was always the same. For example, even when reward was removed in experiment two, the rat was required to visit the opposite end of

the track before the remaining reward was refilled. We observed an overall decreasing rate of forward replays over the course of experiment one which reached significance in epoch 3 (Figure 3.7). This decrease may be a result of the task becoming increasingly perfunctory while the need for planning-related forward replays decreased. On the other hand, reverse replays did not decrease overall in epoch 3, possibly because they are sensitive to reward. Since reward changed in each run, the need for reward learning-related reverse replays was not decreased by epoch 3.

Consistent with a role in planning or decision-making, in tasks involving decision making, replays (Karlsson and Frank, 2009; Singer et al., 2013) and replay-like "forward sweeps" (Johnson and Redish, 2007) are observed at choice points in conjunction with reward expectation signals in the ventral striatum (van der Meer and Redish, 2009). In a spatial memory task, replays can reflect the location of a remembered goal, and predict the behavioral trajectory an animal will take to get there (Pfeiffer and Foster, 2013). Blocking SWRs impairs working memory performance in a spatial task without affecting reference memory (Jadhav et al., 2012). However, our evidence of functional differences between forward and reverse replays, warns of the inadequacy of generalizing replay function. Replay detection and even more so accurate decoding of content requires a large number of simultaneously recorded cells, and many studies have used SWRs as a proxy for replay to bypass this technological hurdle. SWRs do not distinguish replay of the current environment from that of other environments, and do not distinguish direction. The interpretation of several previous studies would be complicated if a substantial number of the replays occurring at the time of experimental manipulation

were reverse replays depicting incoming behaviors, rather than forwards replays depicting planned outgoing behaviors, as presumed.

The meaning of replay rate changes

An interesting issue in the interpretation of our results concerns the fact that reward information is apparently conveyed by the increase or decrease in rate of reverse replay. A very strong interpretation of our results would be to state that reverse replay "encodes" reward magnitude and/or reward prediction error. While possible, this interpretation sits uneasily with other considerations. For example, if reverse replay in the hippocampus is an input signal to downstream circuits that learn to predict rewards (e.g. learn a spatial value function), then increasing replay rate might be expected over time to increase the weights of place cell inputs to value-representing neurons, thereby increasing the magnitude signal conveyed by these neurons. But this is a rather unwieldy mechanism, since the number of learning events will increase without limit for as long as the rat stops in place. Alternatively, downstream circuits might be sensitive specifically to the rate of events – but it is not clear how such a replay-rate-sensitive scheme could be implemented, since the timescale involved is long (seconds). It would be computationally simpler to use separate mechanisms to encode reward magnitude and the spatial trajectory with which the reward is to be associated – for example, by pairing reverse replay sequences with larger or smaller dopamine signals, according to the model proposed in (Foster and Wilson, 2006).

We prefer a more conservative interpretation of our results: that replay rate changes arise out of coordination of reverse replay with dopaminergic or other reward-related signals, and thus the rate changes themselves are merely a necessary by-product of this coordination. Such a mechanism could facilitate synaptic strengthening within the replaying cell ensembles as well as their downstream targets and could lead to increased consolidation via later reactivation. This scheme would, however, necessitate the controlled initiation of reverse replays by an external signal, for which there is already suggestive evidence (McNamara et al., 2014). Hence, we expect that the importance of our results lies in the notion of selective triggering of reverse replay, the suggestion that reverse replay has a unique relationship to the processing of reward, and the demonstration that replay is a heterogeneous phenomenon with forward and reverse events having quite different functions.

Chapter 4: SWR and replay timing during licking behavior

Background

Although sharp wave ripples have been studied for more than 40 years, the behavioral and network conditions that give rise to the precise timing of a SWR or replay event are still unknown. However, SWRs are associated with large irregular activity in the LFP and recent studies have begun to elucidate the neural mechanisms underlying the switch between this state and the theta state. Behaviorally, this switch occurs when the animal transitions between an alert and active state of processing the external world, and an internal processing state. Wang and colleagues have shown that this switch involves a subset of probably glutamatergic neurons in the mesopontine median raphe. Optogenetic stimulation of these neurons results in suppression of SWR activity while inhibition increased SWR rate (Wang et al., 2015). However, these projections do not reach the hippocampus directly so they must affect hippocampal activity through a secondary target.

The medial septum is known to be important for the generation of the theta rhythm in the hippocampus (Lawson and Bland, 1993). Recently this was shown directly with optogenetic techniques. Vandecasteele, et al., showed that selective stimulation of cholinergic septal neurons induced theta oscillations and suppressed ripple occurrence (Vandecasteele et al., 2014). Thus, it may be the inhibition of this population, or competing GABAergic input from the same regions, that mediates ripple occurrence.

Although these studies do not paint a complete picture of the SWR and theta state trade off, they do support theories of neuromodulatory control of brain state (Lee and Dan, 2012). Indeed neuroodulatory systems are good candidates for control of brain state because they project global signals that could coordinate the activity of multiple brain regions.

We have shown that reverse replays are increased at increased reward, which could promote the association between the replayed trajectory and reward. For this to occur, a dopaminergic reward signal would have to be present during the replay. This could be accomplished if dopaminergic input from the VTA could trigger replay, or alternatively if a signal upstream of both the VTA and hippocampus could simultaneously activate both regions. Since dopamine release occurs when an animal consumes reward, and replays were modulated by reward consumption, a common upstream signal could come from a brain region associated with the activity of consuming reward. This could be a motor signal from the tongue or jaw, or a gustatory or olfactory signal from the reward itself.

To indirectly test this hypothesis, we asked if SWR and replay occurrence was more likely while the rat consumed reward. In particular, we wanted to know if SWR or replays would time-lock to discrete actions of consuming reward such as an individual lick during chocolate drink consumption. This experiment is also partially motivated by my observation that SWRs occur frequently while rats are consuming food pellets. I used this tactic to elicit SWRs during tetrode adjusting.

Lickometer

In my initial study of SWRs, replays, and eating behavior, I used slow motion video (iPhone 5 camera) to record the rat's tongue during licking. I extracted the timestamp of each lick by manually searching through the video frame-by-frame to identify the frames in which the rat's tongue entered the reward well. This method is very tedious and time consuming and obviously not ideal for large scale recordings. Therefore I constructed an independent data acquisition system for the detection of licking, or a lickometer. The lickometer takes advantage of the 60-Hz noise in an open circuit to record licking times with millisecond accuracy. One end of the circuit is connected to a wire that is inside of the reward well tube, making contact with the chocolate drink. The other end of the circuit is connected to a foil-covered floor panel in front of the food well on the track. This set-up is shown in figure 4.1.

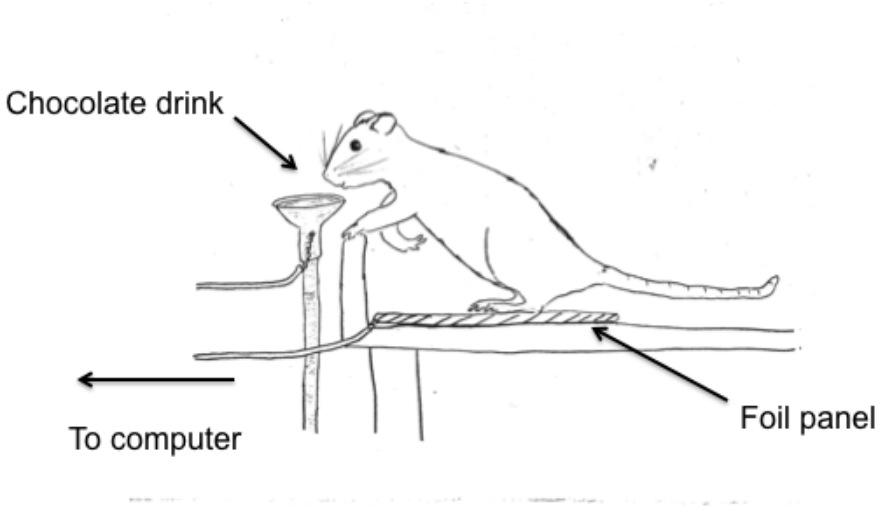

Figure 4.1 Set-up of the lickometer.

One end of the circuit is attached to the foil panel under the rat's feet. The other end of the circuit is in contact with the chocolate solution in the food well. The circuit is

completed when the rat's tongue makes contact with the solution while his feet are in contact with the foil panel. The activity in the circuit is recorded by a custom written Matlab application.

Noise in the open circuit is monitored by a National Instrument Data Acquisition board and recorded in a custom written data acquisition program. When the rat stands at the food well and makes contact with the chocolate drink during licking, the circuit is closed and the noise is canceled out. This signal can then be processed by calculating the square of the z-scored power spectral density resulting in the signal shown in Figure 4.2. Precise lick times can be extracted by thresholding the processed signal and setting maximum and minimum durations allowed for a single lick.

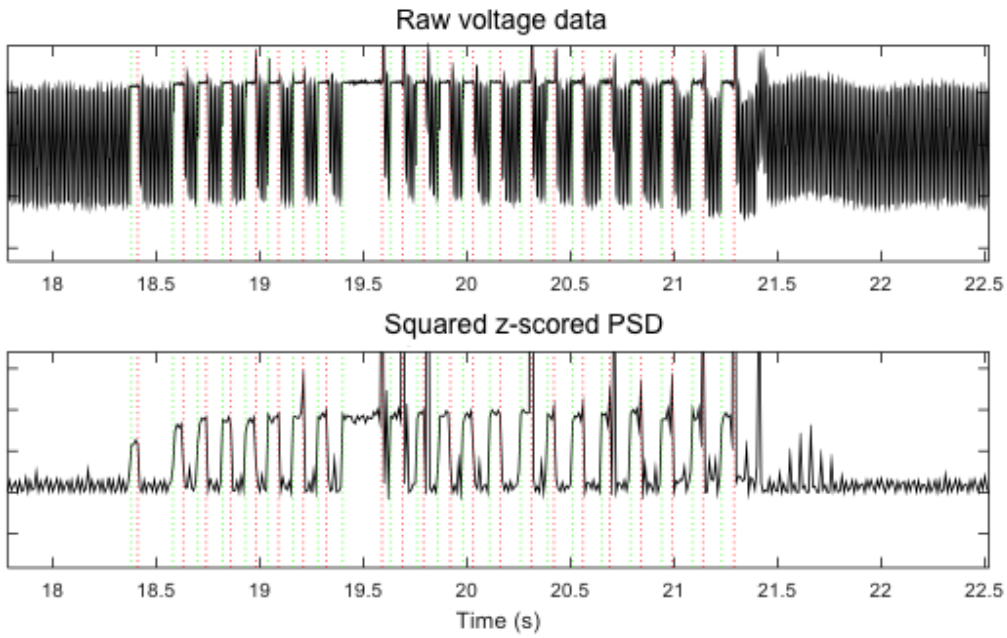

Figure 4.2 Output of lickometer.

The open circuit is sensitive to 60-Hz noise which is cancelled when the circuit is completed (top). The raw signal is processed by z-scoring and squaring the mean power spectral density in the 60-Hz band (bottom). This allows the detection of the onset (green dotted lines) and offset (red dotted lines) of individual licks.

The lickometer acquires data in Matlab while neural data is recorded in Neuralynx. In order to coordinate the timestamps of the two acquisition systems, the lickometer requests the timestamp from Neuralynx when acquisition begins. The lickometer has only been used in recordings from a single animal (W19) and in those experiments the Neuralynx timestamp was acquired before acquisition was started. However, during later analysis I found that starting acquisition was not immediate but occurred with a variable delay of up to 30ms. The delay may be due to the packaging of incoming data

so that acquisition could not be started until the arrival of the next package. In the data presented in this chapter, lick timing may have an error of up to 30ms. This precludes our ability to look for precise timing of SWRs and replays with individual licks, although we can still analyze whether SWRs and replays occur during bouts of licking. The error can be avoided by requesting the timestamp after the onset of acquisition and the current version of the lickometer software does not have this timestamp error.

All lickometer data is from three experimental sessions (see Chapter 3) in one animal, rat W19. Thus, these are preliminary results and should not be construed as descriptive of the entire population.

Results

To determine if SWRs and replays are more frequent around the time of a lick, I calculated the time between each SWR and replay and the nearest lick (before or after). The distribution of SWRs and replays around the nearest lick is plotted in Figure 4.3. Both SWRs and replays peak within a 100-ms window around each lick. This does not necessarily imply that these events occur around licks because licks occur a median of 140-ms apart (at about 7-Hz, roughly the theta frequency). Thus, any event occurring within a bout of licking would be likely to occur within 70-ms of the nearest lick and licking bouts occur during most of the durations of stopping periods.

To calculate the chance level of an event (SWR or replay) occurring during a licking bout, I created a control distribution of events within the same stopping periods. To create this distribution I simulated 1,000 sessions in which I assigned the same numbers of events as observed experimentally to random times within the same stopping periods. I then averaged the distributions from the simulated sessions. The simulated distribution is also shown in Figure 4.3.

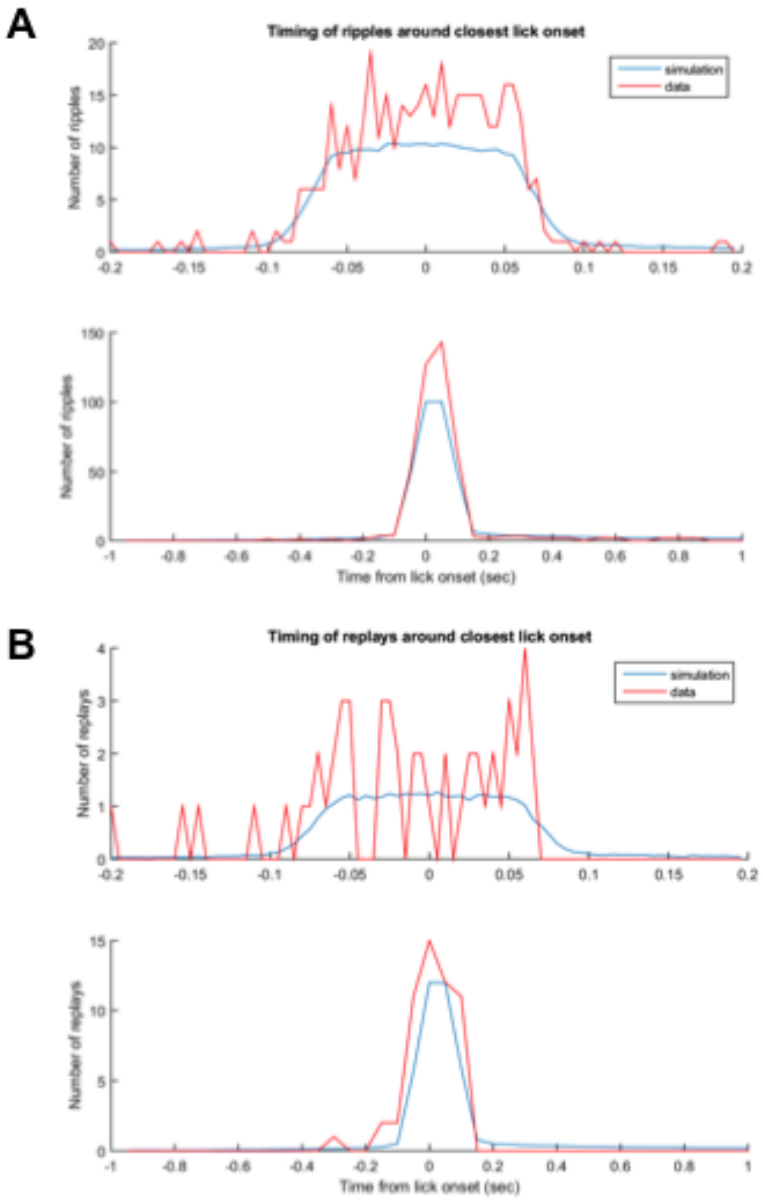

Figure 4.3 Timing of SWRs and replays around lick onset.

(A) Time of SWRs around closest lick onset. (B) Time of bi-directional replays around closest lick onset. Actual data shown in red. Averaged simulated data shown in blue.

The experimental distributions of both SWRs and replays have slightly higher peaks around zero than the simulated distribution. This indicates increased clustering of SWRs

and replays within a bout of licking. To quantify this difference, I fit binomial probability distribution functions to the simulated and experimental data to calculate the probability of an event occurring during a licking bout. An event was described as within a bout of licking if it occurred within 100 ms of a lick. I then performed a binomial test to ask whether the experimentally observed probability of each event occurring during a licking bout was significantly higher than the chance probability. In fact, the probability was significantly higher for all four events, SWRs, bi-directional replays, forward replays, and reverse replays (Figure 4.4).

SWRs occurred during licking bouts 82% of the time (95% CI: 78-86%), compared to the chance level (64%, 95% CI: 63-64%) while 88% of replays occurred during a licking bout (95% CI: 76%-95%). Forward replays occurred during licking bouts 88% of the time (95% CI: 64-99%). Reverse replays were most likely to occur during licking bouts with 92% occurring during bouts (95% CI: 75-99%). These results suggest that a higher percentage of SWRs contain replays during bouts of licking. An average of 13% of SWRs contained replays during licking bouts, while only 7% of SWRs outside of licking bouts contained replays. This difference did not reach significance ($p=0.148$, Wilcoxon rank sum test).

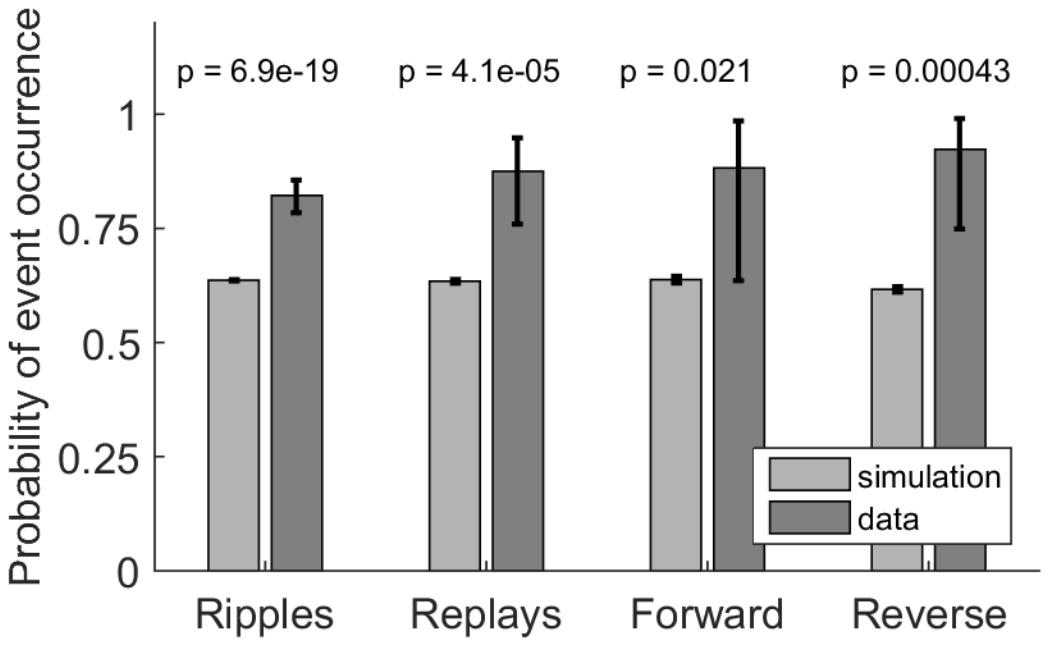

Figure 4.4 SWRs and replays are more likely to occur during bouts of licking. Mean probability and 95% confidence intervals of probability that simulated events (light grey) and observed events (dark grey) occurred during a licking bout.

Discussion

I have presented a custom made system for recording the precise timing of reward consumption. This system will be very useful in any experiments using liquid reward. It is very easy to use and does not interfere with other aspects of the experiment. Since no current is run through the circuit, it does not present a hazard to delicate recording hardware, although grounding the circuit to the Neuralynx box would be wise.

Although SWRs have been associated with a number of activities in rats (Buzsaki, 1986), there is has been little effort to quantify the relationship between SWRs and specific behaviors. Here we present preliminary evidence suggesting SWRs and replays are more likely to occur while the rat is consuming reward. Additional subjects are needed to support this initial evidence. Also, it is clear that while SWRs/replays may be more likely during eating, they can certainly appear at other times. In fact SWRs are strongest and most abundant during sleep. Thus, the relationship between eating and SWR/replay occurrence is only a partial story. However, this result provides some insight into the initiation of SWRs, going beyond the broader category of brain state to identify specific behaviors that may increase the chance that a SWR or replay may occur.

Chapter 5: Online Replay Detecting Algorithm (ORDEAL) for closed loop interaction with hippocampal replay

Background

A significant challenge in the replay field is that it is very difficult to manipulate replay or block replays from occurring. There are a number of reasons for this problem. No physiological or behavioral indicators predict replay occurrence with the millisecond precision required for closed loop interaction with replay. As such, it is impossible to determine the trajectory being replayed until at least part of the replay has already occurred. Previous studies have blocked replays by disrupting hippocampal firing at SWR onset (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009; Jadhav et al., 2012), however this method blocks all SWRs and thus all replays, regardless of content. In our data, we observed that only 15-20% of SWRs co-occurred with confirmed replays of the current environment. This means that up to 85% of events blocked with this method may be either replays of a remote environment, or may not be replays at all.

I designed a closed loop system for online decoding of single unit activity and replay detection and classification. The program is named the Online Replay Detecting Algorithm, or ORDEAL. The purpose of ORDEAL is to detect replays and their content within less than 100ms of replay onset in order to interact with hippocampal function

via brain stimulation. The goal is to trigger brain stimulation during replays of only a certain specified trajectory.

Although there are many possible brain regions and methods of stimulation compatible with ORDEAL, I chose to use ORDEAL to trigger rewarding brain stimulation of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus. The MFB contains axons projecting from many midbrain regions including the ventral tegmental area to targets in the striatum, cortex, and hippocampus. Stimulation of the MFB is incredibly rewarding and rats will lever press for this stimulation for hours on end, as Olds and Milner discovered accidentally in 1954 (Olds and Milner, 1954). The rewarding property of MFB stimulation is most likely mediated by the dopaminergic fibers projecting from the ventral tegmental area, possibly bi-synaptically (Milner, 1991; Wise, 2005).

We hypothesized that pairing rewarding brain stimulation with replay of a certain trajectory would influence the reward association of that trajectory. This could result in a behavioral shift to prefer the replay-rewarded trajectory or an increase in that particular replay signifying increased consolidation of that trajectory. A recent study, in which pairing rewarding brain stimulation with a single place cell's activity increased preference for the field location of that cell in later exploration (de Lavilleon et al., 2015), suggests that behavioral manipulation is possible. Due to challenges, which I will discuss in a later section, we have not yet been able to test our hypothesis in a satisfactory manner. Instead I will focus on the experiment and the technical advances

made on ORDEAL in order to aid the continuation of this work and the development of the ORDEAL program.

Experimental design and methods

I adapted the standard 40 tetrode drive design to contain an individually adjustable bipolar stimulating electrode targeting the MFB. The MFB is ventral to the hippocampus, so the stimulating electrode was lowered along the medial edge of the same craniotomy as the tetrode bundle targeting -4.2A/P, +2M/L, -8.2D/V. The stimulating electrode was constructed from two coated stainless steel 0.0045-inch electrodes (A-M Systems, Inc.) twisted together. The stimulating electrode was lowered 8-mm ventrally during surgery and then fine adjustments were made to move the electrode to its final position. Electrical stimulation was delivered in 300-ms trains of biphasic pulses delivered at a frequency of 100-Hz and amplitude of 100-150-μA. Spike trains were generated by an A310 Accupulser (World Precision Instruments) and an A385 Stimulus Isolator (World Precision Instruments).

Stimulating electrode location and stimulation parameters were adjusted based on the animal's behavioral response to reward. MFB stimulation causes an increase in exploratory behavior and whisking. These behaviors are accompanied by a transient increase in theta range power in the local field potential (Figure 5.1). Since the stimulation is rewarding, rats can be quickly trained to perform a simple task in exchange for reward. I trained rats to bite the end of a metal object in exchange for MFB

stimulation. By counting number of times they bit the tool in 2min periods during which the object was available I was able to judge whether training had occurred. As a control I also measured the number of times the rat bit a visually distinct metal object in the same amount of time. Rats do not naturally enjoy biting a metal object and they learned within minutes the difference between the two objects and stopped biting the unrewarded object very quickly (Figure 5.2). On the other hand, the rats bit the rewarded object almost constantly while it was available. This behavior is very obvious and not elicited by stimulation of nearby regions, so it was deemed sufficient to confirm that the stimulating electrode was located in the correct location. Electrode location was later confirmed by histology (Figure 5.3).

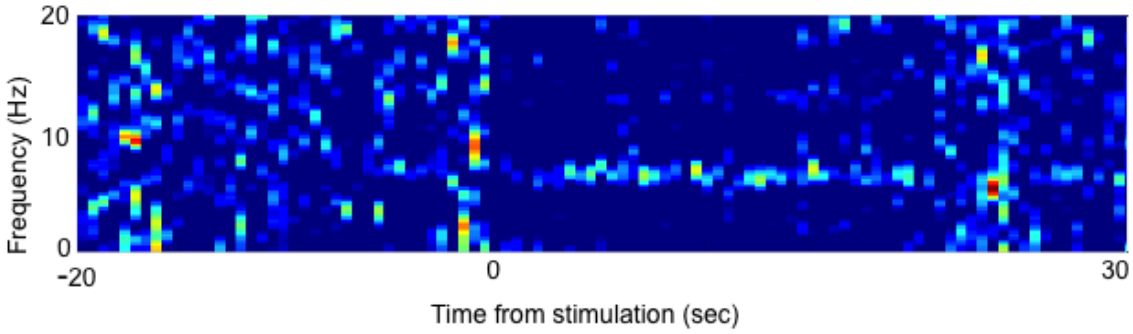

Figure 5.1 Theta power increases following MFB stimulation.

Z-scored power around the time of stimulation onset is shown in color.

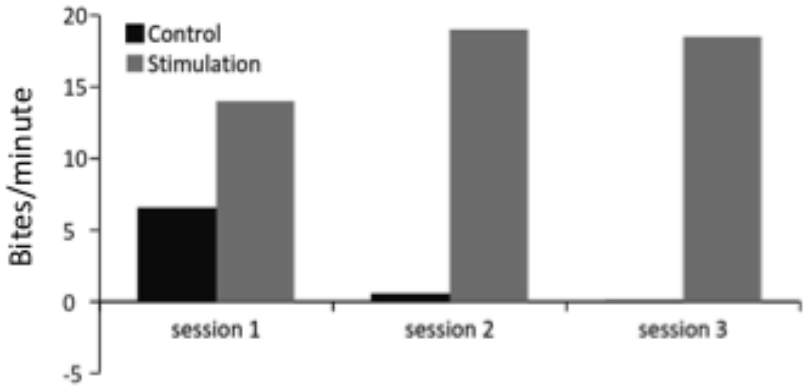

Figure 5.2 Rats can be trained to bite a metal object to obtain MFB stimulation.

Number of bites per minute on a metal object that was reinforced with MFB stimulation (grey), versus an unrewarded object (black) across three consecutive sessions. Data from rat W14.

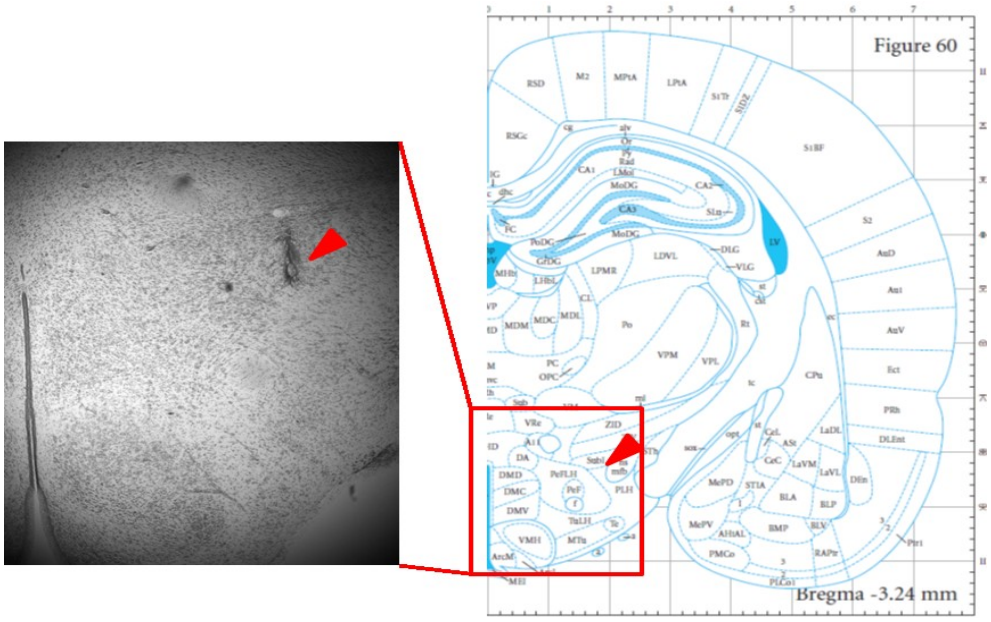

Figure 5.3 Location of stimulating electrodes in the MFB in rat W10.

Rats were trained to traverse a linear track for chocolate reward as described in Chapter 2, and experiments took place on a V shaped maze with reward wells available on each end and the vertex of the maze. The animal was initially placed on the vertex of the maze and allowed to freely explore both arms. On the first exposure to the maze, Run 1, the initialization phase, rats were encouraged to alternate between the left and right arms so chocolate reward was available only when the animal alternated correctly. The initialization phase lasted for 15-mins or the time it took for the rat to visit each arm 5 times. Then the animal was returned to the sleep box for 10-15-mins while ORDEAL was initialized (see next section). On Run 2, the rat was again placed on the vertex of the maze and allowed to explore freely, but this time reward was available on both ends and the vertex of the maze at all time. The run session usually lasted about 30-mins. Replay detection with ORDEAL only occurred when the rat was located at the vertex of the maze. Since around 80% of replays are locally initiated, the replays occurring at the vertex would most often represent trajectories moving from the vertex to the left or right end of the track. ORDEAL allows the experimenter to select which trajectory to use to trigger stimulation. In order to increase the chance of stimulation reaching the brain before termination of replay, the arms of the track were made at long as could be fit into the experiment room, 2-m each. I also recorded 30-mins in the sleep box before Run 1, and at least 1-hr on the sleep box after Run 2.

ORDEAL

Online replay detection occurs in two steps. In step 1, initialization, place units are automatically sorted and defined, place fields are established, and the user can select the parameters of the program. In detection, step 2, a continuous stream of spike data is downloaded from the Neuralynx recording software, incoming spikes are sorted into existing units, Bayesian decoding is performed, replays are detected, and stimulation is triggered upon detection. The user interface for ORDEAL is shown in Figure 5.4.

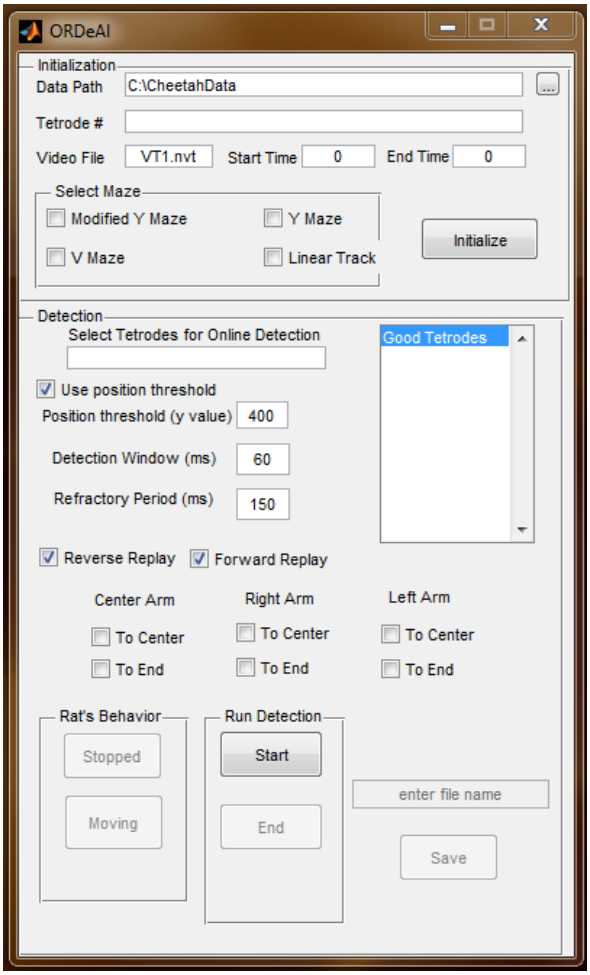

Figure 5.4 GUI of ORDEAL

The initialization step utilizes the spike data recorded in Run 1, and occurs while the rat is on the sleep box between Runs 1 and 2. First, the user specifies location of the spike data, the tetrodes to be used, the title of the video file, and the start and end times of Run 1 in seconds. Before initialization begins, the user must select the maze that recording took place on. This is required to linearize the rat's position appropriately. I included four options: Y shaped, modified Y (Wu and Foster, 2013), V shaped, and linear. Only the V shaped and linear track options have been used, but the Y maze options were included to allow expansion to different experimental designs. Once all of these variables are set the user can click on "Initialize".

First, spike data from the selected tetrodes are automatically clustered using a custom written program (Winclust, David Foster). Tetrodes are selected by the experimenter based on the quality of isolated units on those tetrodes. It is unfeasible to use all tetrodes at this step because the runtime of Winclust can become very long, especially when attempting to cluster poorly isolated units. The duration of Run 1 is also limited by the amount of data that can be analyzed by Winclust in a reasonable amount of time. I chose to use the amount of data that can be initialized in 15-mins. Once spikes have been assigned to clusters, ORDEAL calculates the mean and standard deviation of each cluster. These values are used as parameters of a multivariate normal distribution approximating the location of the cluster. Incoming spikes are later assigned to a unit based on their probability of belonging to the multivariate normal distribution.

Once units are defined, place fields for each unit are calculated as described in Chapter 2. As a test of place cell and decoding quality, ORDEAL outputs the Bayesian decoding of position in Run 1 as the final step of the initialization phase. This allows the experimenter to gauge whether decoding is accurate enough to continue the experiment. However, our lab has also shown that good position decoding does not necessarily predict good replay decoding (Altimus, et al., SFN abstract 2015).

After initialization the user has the option to select a subset of tetrodes to use in the detection phase. This is important because the slowest step in replay detection is downloading spike data from Neuralynx to Matlab, so using all tetrodes may slow down detection too much. The user can also specify the position threshold on the rat's location, which runs ORDEAL only when the rat is at the specified area on the maze. If "use position threshold" is not selected, the user can manually turn ORDEAL on and off using the "Stopped" and "Moving" buttons. ORDEAL is on when the rat's behavior is set to "Stopped". The detection window, or the size window to use when decoding, can also be specified as well as the refractory period, or time after stimulation is delivered that a second stimulation cannot be triggered. Finally, the specific replay content (arm and direction) can be specified. Forward and reverse replay detection is not yet enabled.

When set up is complete, the user can start replay detection. This opens a connection with Neuralynx, downloads new data from the specified tetrodes, and keeps a running buffer of spike activity. New data is downloaded continuously. On each iteration the most recent data in a time window of the size defined in the detection window variable

is decoded using the Bayesian method discussed in Chapter 2. The decoded window is then tested with weighted correlation, template matching, or using probability density. If the chosen measure exceeds threshold, an output signal is sent to a National Instrument Data Acquisition board, which triggers stimulation. When replay detection is stopped the save button is enabled and times of stimulation can be saved.

Challenges and potential improvements

A major challenge to the accuracy of ORDEAL is how to sort incoming spikes into existing clusters. In offline tests, by-hand clustering, automatic clustering, and the multivariate normal distribution based spike sorting described in the previous section yield similar decoding and good decoding of replays (Figure 5.5). However, in the offline spike sorting method, the normal distribution was defined from the entire session instead of a short initialization period. Perhaps a longer initialization period would allow for better estimates of cluster parameters. However, if recording is even slightly unstable and spike amplitudes increase or decrease over the recording session, then spike sorting accuracy will rapidly decrease. A possible improvement to this method would allow cluster location to be updated as new spikes are recorded.

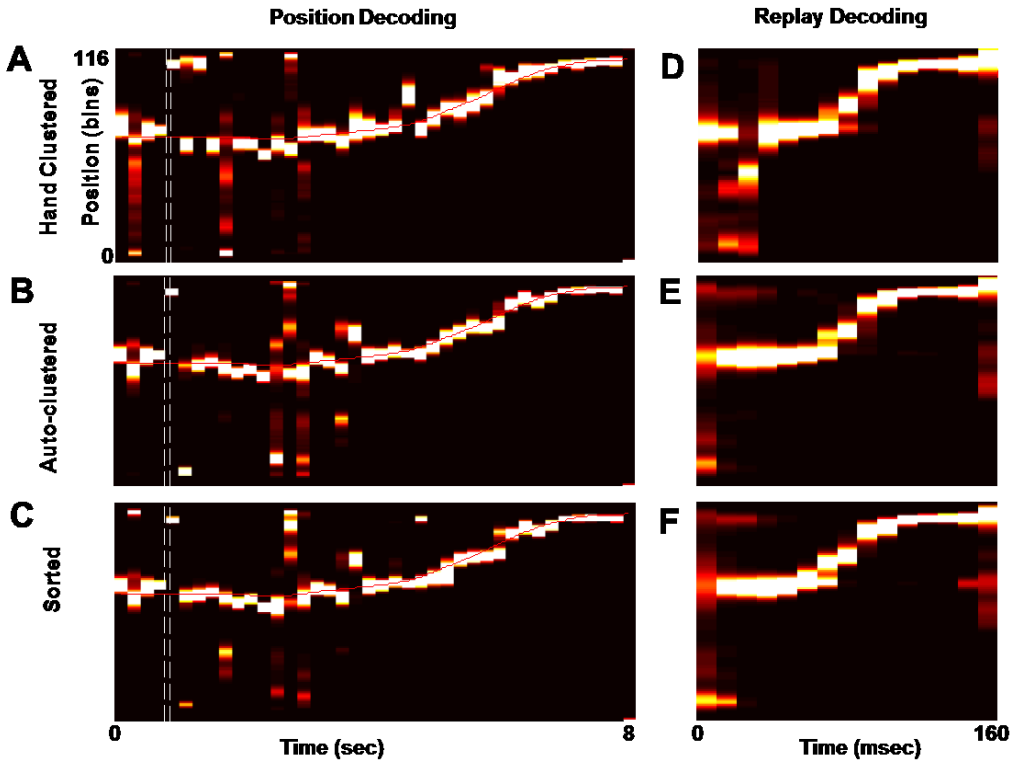

Figure 5.5 Decoding based on three methods of unit clustering.

Position decoding is shown on the left with actual position overlaid in red, replay decoding within the window outlines in white is shown on the right. By hand clustering with xclust (top), auto-clustering with winclust (middle), and spike sorting using multivariate normal distribution defined from auto-clustered units (bottom).

Another constant challenge is the speed of replay detection. As previously mentioned, this is limited by the number of incoming spikes that are downloaded on each iteration of the program. Because each iteration downloads all data acquired since the previous iteration, longer time to download new spikes results in a higher number of new spikes waiting to download in the next iteration. This creates a positive feedback cycle that can

cause the program to fall seconds behind if too much data is fed in to it. A careful analysis of the limit of data that can be processed without compromising speed is needed. It would also be useful to build in an estimate of the amount of data being recorded on each tetrode as part of the initialization to allow smarter tetrode selection for the detection step.

Methods of replay detection can also be improved on. Offline methods which are already imperfect are even less relevant in online detection. First of all, decoding is messier online due to the spike sorting issues. Additionally, decoding is done in a time window that is shorter than a replay and may begin tens of milliseconds before or after the replay onset. A potential solution to this problem is using peaks in ripple filtered LFP or multi-unit activity to trigger decoding, thereby increasing the change of decoding a replay and catching the beginning of the replay in the decoded window. There are also many ways to detect replays, including the three I have mentioned, and better ways can always be devised.

ORDEAL accuracy was quantified by comparing online detection to the offline decoding of confirmed replays. This allowed me to calculate rates of true positives, false positives, and false negatives. Unfortunately the rate of true positive detection was very low, on average less than 10%, while false positives were very high. Figure 5.6 shows three examples of events which ORDEAL identified as replays based on weighted correlation. The first event has highly concentrated probability along the trajectory and coincides with a SWR and is likely a true positive. The second and third events do not occur with

SWRs, but do have trajectory-like decoding. Cases like these may be false positives, however their classification is more ambiguous.

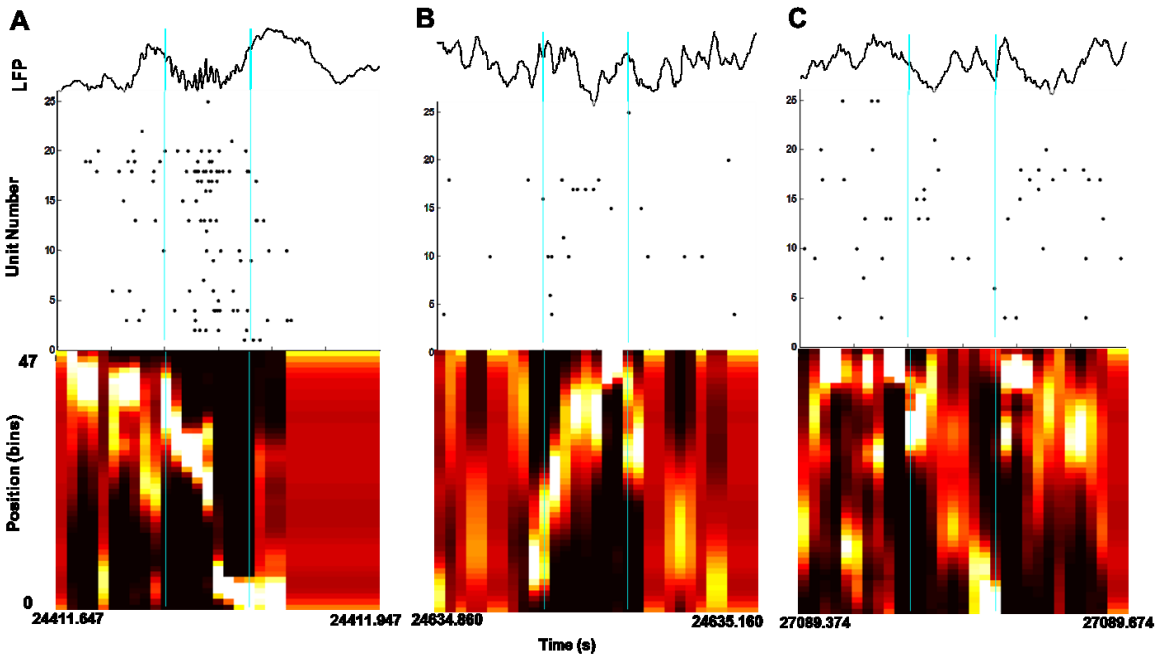

Figure 5.6 Examples of replays detected by ORDEAL.

Raw local field potential (top), raster plot of 25 cells ordered by place field locations on the track (middle), Bayesian decoding (bottom). Cyan lines denote the boundaries of the replay detection window. (A) A good example of SWR and replay. (B) A potential replay event. Note the relatively lower spiking outside of the event. (C) A second potential replay event which may be noise. Note that spiking is frequent outside of this event.

This ambiguity is perhaps the greatest challenge to evaluating the ORDEAL program. Due to different processing methods, ORDEAL often picks up replays that are not

confirmed by offline analysis. These replays are classified as false positives. In general, offline replay detection is more efficient than online detection, however offline detection is limited by the number and quality of place cells recorded and there is no gold standard of replay detection with which to compare ORDEAL's performance. Thus, it is impossible to know if false positive replays detected by ORDEAL are true errors. This is an important caveat to remember when evaluating ORDEAL accuracy.

Discussion

Although online replay detection presents a significant technical challenge, it will be an important piece of technology in the hippocampal replay field. ORDEAL will require further development, but it provides an advanced framework for detecting and manipulating replays. It can also be expanded on to fit different experiments. For example, if we wish to interrupt replay, then we must develop a way to detect replay within the first tens of milliseconds. This may require deviation from the Bayesian decoding method I have used in my algorithm. It is also possible to adjust the durations of replays to an extent by changing the length of the track, which makes early detection easier. Additionally, ORDEAL is not limited to electrical stimulation and can easily integrate with optogenetic methods, which have become standard.

Steps towards interacting with replay have already been taken. de Lavilleon and colleagues triggered rewarding brain stimulation in response to the activity of a single place cell during sleep. This manipulation resulted in increased time spent in the field of

the rewarded cell during subsequent behavioral testing (de Lavilleon, et al., 2015). Detecting the activity of a single neuron is much more straightforward than detecting replay but this study suggests that closed loop interaction with hippocampal activity can yield interesting results and even influence behavior. These experiments aim to artificially associate reward with a previously formed memory or context. The ability to retroactively change the emotional association of a memory could eventually lead to treatments of emotional disorders like post-traumatic stress disorder.

Regardless of whether my particular experiment is completed, it is clear that the hippocampal replay field is in need of causal evidence for the function of replay. For any of these studies to occur, some technological developments along the lines of online replay detection will be necessary. I hope that the work I have done thus far will be a basis for future experiments. ORDEAL will allow us to determine the specific contribution of replay to spatial learning and planning, and investigate the interaction between the hippocampus and other areas of the brain involved in learning and memory.

Chapter 6: General Discussion

Translating experience into memory is the function of the hippocampus. Yet some experiences are remembered more vividly than others, particularly experiences with strong emotional associations. Numerous hypotheses exist to account for this memory enhancement, including emotionally induced attention or arousal states, novelty, salience, and increased mental rehearsal of emotionally charged events (Cahill and McGaugh, 1998). While these factors may involve or alter hippocampal function, this has not been studied at the level of single unit hippocampal activity. Additionally, much of the research in this field in both animals and humans focuses on the effects or either stress hormones or fear related amygdala activation on memory (Cahill and McGaugh, 1998; Dolcos et al., 2004). These are relevant topics given their implications for post-traumatic stress disorder and other mental illnesses in which negative memories become so strong as to be overwhelming and debilitating. However, positive emotions also enhance memory, and studying the mechanisms underlying this phenomenon may inform understanding of emotional memory processing in general.

We have shown that reward modulates replay, a hippocampal mechanism of memory formation and storage. Although it is difficult to gauge the emotions of a rat, it is generally accepted that for a hungry rat, receiving food is a positive experience associated with dopamine release (Wise, 2006). We manipulated the magnitude of food reward to test the response of hippocampal replay. When reward increased, the rate of reverse replays representing the preceding trajectory was increased as well. Replays can

facilitate the strengthening of synaptic connections within the activated ensemble and their downstream connections, which strengthens the representation of the replayed trajectory (Carr et al., 2011). Increased reverse replay at a reward location would facilitate the creation of a stronger than average memory of the trajectory that led to the reward. On the other hand, forward replays were unaffected by our experimental conditions. We speculate that this is because awake forward replays are involved in planning which is not an important part of the linear track task.

How does replay support learning not just the context of the episode but also the reward distribution within the context? Our hint that replay also supports learning the reward structure of the environment came from the observation that only replays in the reverse direction were modulated by changing reward. This is significant on both an intuitive and a mechanistic level. Intuitively, the structure of reverse replay is consistent with the retrospective behavior we associate with learning. It is the neural re-tracing of one's steps to a relevant past event, for example the choice that led to reward, although it occurs on a timescale that is too fast to be consciously experienced. Mechanistically, reverse replay could be paired with a dopamine signal that would assign value to the replayed locations. Dopamine neurons in the ventral tegmental area respond to reward with phasic (burst) firing, resulting in global dopamine release that decays over the course of hundreds of milliseconds to seconds (Cheer et al., 2007). Pairing a replay in the reverse direction with this signal would result in a decaying reward association as the trajectory moved further from the reward location (Foster and Wilson, 2006).

Through this mechanism, the locations in a cognitive map could gain graded value associations with the earliest replayed positions weighted more strongly than the later positions. Increasing value associations would be unsustainable unless the cognitive map had some instability or uncertainty, perhaps leading to decay in the value associations across time. Under these conditions, a baseline amount of replay may be necessary to maintain the map, while increases and decreases adjust value associations respectively. We found that changes in reverse replay adaptively code the relative reward magnitudes available in the environment and reflect changes in reward. Thus when reverse replays increase with reward, the value association of the trajectory leading to reward is increased, but when reverse replays are decreased the reward association decays. This mechanism is consistent with the observed scaling of replays between the increased and unchanged ends of the track. It also explains how the rat could learn from the absence of reward.

A more conservative theory to explain our results is simply to say that reward learning occurs through the coordination of dopamine and reverse replay. The coordination with reverse replay could be mediated by direct effect of hippocampal dopamine release or a common upstream signal to both the hippocampus and ventral tegmental area. The result of this coordination is that the changes in reverse replay rate mirror dopamine responses to reward. Dopamine neurons respond most strongly to unexpected reward (Schultz, 1997). We observed that reverse replays had a tendency to adapt to the present reward magnitude similarly to dopamine neurons (Tobler et al., 2005) and we suggest that reverse replays are paired with dopamine release and thus may follow the same

trends as dopamine release. In our experiments, the rats always received chocolate on both ends of the track (except in the no reward condition). Therefore, in the baseline state we might expect little dopaminergic activity when reward is received. When reward was increased at one end of the track, this unexpected additional reward could lead to the increase in reverse replay. However, when the reward decreases back to the baseline amount in run 3, the rat actually receives less reward than expected in this location, and we observe a decrease in reverse replay relative to baseline.

Withholding expected reward is known to decrease dopamine neuron firing, and similarly, reverse replays were decreased to a lower than baseline level in run 3 in experiment one. On the other hand, removing reward did not lead to an increase in reverse replays at the opposite end of the track in experiment two, perhaps because the reward on that end of the track was unchanged from run 1 and thus not unexpected. However, when reward is returned in run 3 we observed an increase in reverse replays to higher than baseline levels at the same time that we would expect an increase in dopamine release.

These conclusions assume that the unexpectedness of reward does not significantly change over the 15 laps in each run. We did not observe any changes within a run when considering lap within run as an additional variable in our models. This may seem unlikely, but actually many training protocols require much more than 15 trials or occur over a number of days. For example, training rats to lever press for food pellets takes around 45 minutes and upwards of 150 responses (Wise and Schwartz, 1981). Any

experience related changes in reward response may be undetectable in a 15 trial period, especially when the subject knows the task to be inherently unpredictable, as may be the case with animals that have experienced multiple sessions of the reward manipulation experiment.

In this dissertation I have introduced two technical advances, the lickometer and ORDEAL, which can be used for future experiments. I tried to make these applications user-friendly and I hope that they will be used. Both programs can be easily modified (either in the code or in the hardware) so they have a range of applications. The hippocampal replay field is at a point where technological innovations are becoming commonplace and the development of custom software to control increasingly complicated experiments is necessary.

We have discussed the importance of carefully controlling for behavior in these experiments (Chapter 3). The lickometer will make this much easier because it allows the experimenter to know the exact timing of licking onset, offset, and duration. This is particularly important since we have found preliminary evidence that SWRs and replays are increased during eating. Licking information is usually inferred from overhead camera data, but this method is much less precise. Furthermore, the lickometer will allow for correlations to be made between SWRs/replays and the precise time of licking behavior. The lickometer is easy to set-up and can acquire data simultaneously with Neuralynx and does not interfere with electrophysiological recording, so licking data can be acquired with minimal effect on any experiment using liquid reward.

Many studies have explored the characteristics and limitations of replays, but specific causal effects have been more difficult to come by. Past studies have been limited to interfering with SWRs globally, rather than dissecting the contributions of specific replays to behavior. Additionally, results have been mixed. Blocking awake SWRs in an alternation task affected working memory (Jadhav et al., 2012) while blocking sleep SWRs interfered with consolidation (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009). The results I have presented suggest that the effect of blocking awake SWRs is due to blocking forward replays. It would be interesting to know whether forward replays would be increased on the alternation task and if blocking forward replays only could yield the same result.

Until methods are developed for the direct manipulation or blockage of replays, all hypotheses of replay function remain correlative. ORDEAL is a highly developed application for online replay detection that with some improvement would be a valuable tool. I have discussed many of the technical challenges that will have to be overcome, with the hope that this information will be useful in the continuation of the ORDEAL project. Some shortcuts may have to be taken around the perfect detection of replay, and some level of false hits will have to be tolerated. However, online replay detection is going to be very important in the hippocampal replay field.

Future directions

A weakness of this study is the confound between reward value and reward quantity. Increased reward quantity means that the animal takes longer to consume the reward, resulting in more time spent at that reward location. We have shown that the increase in reverse replays is an increase in rate, which we assume is independent of the amount of time stopped. However, we would prefer not to rely on such assumptions. To disentangle reward quantity with reward value, further experiments should use different types of reward of varying value. For example, using sucrose solutions with increasing sugar concentration could be a good alternative. Furthermore, we have shown that removing reward entirely does result in a significant behavioral change. Instead, it would be better to decrease reward value without changing the amount of food present. In all of these experiments the reward would need to be calibrated so that the animal would not lose interest in the lesser reward and cease to consume it. In my experiments, I limited a run session to 15 runs because after that point the rats began to be satiated and sometimes skipped reward leading to the behavioral confound of the no reward situation.

Although I have claimed that reverse replays are related to reward learning and forward are related to planning, there remain many experiments that would test this theory. My experiment took place in the most basic environment in which replays are studied: the linear track. Additional results would be obtained from a similar experiment in any other environment or behavior task.

We could explicitly test whether the increase in reverse replay at increased reward is associated with the extent of reward learning. For example an animal could be trained to find multiple rewards of different sizes in an open field or "crossword" maze. In such experiments, learning is measured as place preference during probe trials. One could expect the rat to spend the most time in the location of the greatest reward and to find a correlation between reverse replays representing paths to a location and number of approaches to that location. Another experiment would be to block reverse replays specifically at one reward location to test the effect on memory of that location. This could be accomplished using an improved version of ORDEAL.

We have also suggested that forward replay may be a mechanism of memory retrieval or planning. It is unclear whether it is one or both of these functions. Planning would imply that replays would predict the rat's future behavior, yet specific replayed trajectories do not determine the exact path an animal will take (Pfeiffer and Foster, 2013). Instead, forward replays could provide mental exploration of an environment to inform future behavior while actual behavior is flexible. For example there is some evidence that replays can stitch together previously experienced paths into novel paths and use those paths to find shortcuts within an environment. Additional evidence that forward replays specifically could generate novel paths on tracks or fields with potential shortcut routes would strongly suggest that forward replays contribute to behavioral guidance.

Particularly, it would be interesting to test a combination of planning and reward value processing functions on a maze with one or multiple choice points. For example, the rat could be required to run in a certain sequence on a maze with three or more arms, in which the relative value of the reward on each arm is different. Or, the rat could perform this task for equal reward at all locations, but better rewards could be given randomly. This would dissociate the location of the better reward from the planning aspect of the task, although it would require the location of the better reward to move. If the better reward location is dynamic it is possible that the rat might not associate it with any location because of its uncertainty. However, I would expect an increase in reverse replays at the randomly increased reward for two reasons. First, I think that reverse replay is linked to dopamine release, which is known to be strongest in the presence of unexpected rewards, and second, because most learning is formed from a single experience and so it must be able to happen quickly. The most interesting behavioral experiment would take place in an open field, where behavior and replays are not constrained to a track. Unfortunately, it would be difficult to do these experiments because it is challenging to encourage rats to explore the field thoroughly enough for sufficient sampling of place fields to decode replay directionality.

Recently, a few studies have utilized dual single unit recordings in the hippocampus and reward processing areas such as the ventral tegmental area, ventral striatum, and neocortex. This kind of multi-region recording will be key to understanding the interaction between these areas, particularly during replay. Past work has relied on oscillatory synchrony of local field potential across regions which is useful for

establishing a functional connection between regions (Buzsaki, 1996; Fujisawa and Buzsaki, 2011) or between cell pairs in different regions (Gomperts et al., 2015; Lansink et al., 2009; van der Meer et al., 2010). However, simultaneous monitoring of replay events and spike activity in other regions will help us understand how reward related activity is influenced by replay and vis versa.

We have shown heterogeneity in replay directionality, but whether replays can be broken down even further by content is still unknown. When we run our replays through the Bayesian decoding, the output only tells us about the parameters we put in, position and direction. Decoding replays using only position results in beautiful sequences, however, it is impossible to tell the heading direction of the replay. Could there be more parameters, for example sensory inputs, motivation, satiation, or non-running behaviors, that are encoded in replays, but whose code we don't know? Are two reverse replays with identical Bayesian decoding actually representing completely different experiences? And are forward and reverse replays encoded in different subpopulations of neurons, or do they overlap?

I have started to ask these questions by analyzing the subsets of cells that are active in different replays of the same trajectory. It is possible that this analysis could reveal sub-structure in replay events beyond the positional and directional components and the trajectory-like Bayesian decoding. If different subsets of cells are active in different replays of the same environment, it is possible that many of the SWRs we detect which do not appear to contain replay, would contain replay if we were recording from the

appropriate subset of cells. Perhaps we can even find similarities between non-replay SWRs that would suggest those SWRs are replaying the same event. Also, what are the implications for hippocampal circuits within CA1 and CA3 if we do find the existence of functional subsets of neurons? And if there are no subsets—if all cells are equally likely to participate in every replay—what would this mean at an anatomical level? Many of these subtleties can be missed when we process our single unit data through the Bayesian algorithm, and it is important for us to leverage the full information content of our data sets with this type of analysis. I think it will yield interesting results about the generation and structure of replay and help us understand what is going on in non-replay SWRs.

Conclusion

Reward has traditionally been studied in terms of associative learning through conditioning experiments. In these experiments, the subject receives reward following the presentation of a discrete stimulus (classical conditioning) sometimes with the additional requirement that the animal perform a certain action (operant conditioning). Learning is measured as the transfer of a neural or behavioral response from the reward itself to the cue that predicts reward (Wise, 2006). Similar experiments measure learning in response to aversive cues. However, it seems impossible that cue-response associations alone could support all of the complex, value-based decisions that guide human behavior. On the other hand, declarative memory and spatial memory have focused on the hippocampus, which provides a contextual framework to support

memory (O'Keefe and Dostrovsky, 1971; Squire and Zola-Morgan, 1991). Yet how the hippocampus is able to integrate abstract information such as reward or emotion is still an open question.

In this work, I have attempted to reconcile these two memory systems by showing a mechanism by which reward information is integrated with the neural representation of experience. We have seen that changing reward, and possibly just the act of consuming reward, alters SWR and replay activity in the hippocampus. Reverse replays are modulated by reward, thereby singling out a specific event to be coordinated with reward related activity. Still, there is a long way to go in understanding how these changes support the processing of experience into memory. That is why studying the hippocampus, and its key electrophysiological features including (but not limited to) SWRs and replays, and their interaction with reward is so important.

O'Keefe and Nadel extended the cognitive map theory in humans to include not only spatial maps but also not purely spatial maps which support abstraction of thought and language (O'Keefe and Nadel, 1978). I propose an extension of the traditional theory of the hippocampus to include a map of the internal world of emotional associations and value judgments in addition to spatial and linguistic maps. I emphasize that this extended cognitive map, perhaps better described as a network to separate it from a purely spatial interpretation, is defined by personal experience and results in the unique set of perceived principles that guide an individual's behavior. Support for this idea has recently gained ground as new attempts are made to reconcile divergent lines of

hippocampal research in humans and animals (Schiller et al., 2015). Value associations are shaped by our particular circumstances and shift constantly throughout our lifetimes as we learn from mistakes and change our perspectives.

Decisions are made based on multiple factors and previous experience that integrates into a single value estimate. This value is then compared to a threshold to determine, either consciously or unconsciously, if the action should be taken. Consider the decision whether or not to attend college. This decision hinges on factors such as higher future earnings and better job satisfaction, but also student debt, moving away from family, and taking longer to enter the workforce. These factors have no objective values. Instead the values are uniquely determined by the culmination of a person's particular experiences and idiosyncratic cognitive map of value space.

This view of behavior selection accounts for individual differences in decision making without assuming that bad decisions are inherently unintelligent. Excessively negative or positive experiences can contribute to an unbalanced worldview and a skewed sense of judgment. Under these circumstances bad decisions may result from lack of experience and inability to accept or know possible outcomes rather than a willful disregard of consequence. This is a rather radical, but not entirely new concept. In fact, Tolman argued for the importance of building balanced or, in his words, broad, cognitive maps through a balance of motivation and frustration in his original 1948 article. In his concluding remarks he suggests that for the improvement of humanity:

"We must, in short, subject our children and ourselves (as the kindly experimenter would his rats) to the optimal conditions of moderate motivation and of an absence of unnecessary frustrations, whenever we put them and ourselves before that great God-given maze which is our human world. I cannot predict whether or not we will be able, or be allowed, to do this; but I can say that, only insofar as we are able and are allowed, have we cause for hope." – E.C.Tolman, 1948

To truly understand the basis of human behavior, even in complicated decision making, we need to understand how the brain compiles its constant flow of information into the psychological principles that govern our lives. If we think of behavior in this way, we learn to empathize and to reserve judgment on actions that do not make sense to us. We begin to understand that behavior is a consequence of experience and that access to broad experiences through education, diverse communities, and opportunities are essential for the development of Tolman's broad cognitive maps. The hippocampus and the reward system are critical for processing experience and developing these maps and so they are central to this theory. To me, this is why the study of the hippocampus is crucial not just for the scientific field but to all manners of social and health related fields that seek to understand or change human behavior.

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Curriculum Vitae

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Research Publications

Ambrose, R.E., Pfeiffer, B.E., Foster, D.J. Reverse replay of hippocampal place cells is unique modulated by changing reward. *In revison*.

Hashemi, P., Dankoski, E.C., Wood, K.M., **Ambrose, R.E**., Wightman, R.M. In vivo electrochemical evidence for simultaneous 5-HT and histamine release in the rat substanstia nigra pars reticulata following medial forebrain stimulation. *J. Neurochem*. 2011; 118(5), 749-759.

Conference Presentations

Ambrose, R.E., Pfeiffer, B.E., Foster, D.J. Reverse replay of hippocampal place cells is uniquely modulated by changing reward. Society for Neuroscience Annual Meeting, 2015.

Pfeiffer, B.E., **Ambrose, R.E.**, Foster, D.J. Hippocampal replay during an open field spatial task. Society for Neuroscience Annual Meeting, 2011.